THE APPLICATION OF CERTAIN PHYSICO-CHEMICAL PRINCIPLES TO THE PRODUCTION OF STERILE PHARMACEUTICAL SOLUTIONS.

BY PETER MASUCCI.

The new science of chemo-therapy had its origin and its greatest stimulus in the epoch-making researches of the distinguished chemist and biologist, Paul Ehrlich. The world owes to this genius its conception of the principles of biochemical affinities, as well as the development of one of the most remarkable synthetic compounds known to man—salvarsan or arsphenamine. This development was based on well-established chemical laws: the laws of mass action, concentration, molecular constitution and pharmacodynamic effects.

Since the publication of Ehrlich's researches, investigators have devoted their attention more and more to the application of physico-chemical laws to the study of the living organism. The great strides made in colloid research and its application to biology is a typical illustration. More recently the determination of hydrogen-ion concentration of solutions and its influence on biochemical compounds has occupied the time of many research workers.

In the preparation of sterile pharmaceutical solutions intended for subcutaneous or intravenous use, the same physico-chemical factors must be considered, in order to obtain solutions that are therapeutically active, safe to administer, the least irritant and the most stable in market containers.

The importance of testing the various salts for identity, purity, and strength is unquestioned but that is not sufficient. Other factors must be given serious consideration, especially with solutions that are to be injected directly into the blood stream. This so-called intravenous therapy is gradually acquiring many adherents, and in some cases has proved far more efficacious than the more familiar subcutaneous method.

Among the more important factors entering into the preparation of sterile pharmaceutical solutions intended for subcutaneous or intravenous use are (*a*) the concentration which causes the least reaction or irritation locally or systemically, (*b*) the hydrogen-ion concentration and means of buffering the solution, and (*c*) the methods of sterilization by heat and what degree of heat, or by candle-filtration. The importance of each of these factors will be elaborated upon and examples given to illustrate the various points.

CONCENTRATION.

While the proper dosage of drugs has been well determined in the vast majority of cases, little attention has been paid to the proper concentration for injection. It must be borne in mind that when a solution of a drug is administered subcutaneously or intravenously, a chemical action takes place between the drug and certain cells of the body. This reaction obeys the laws of chemical dynamics in exactly the same manner that two chemically active substances do when mixed in a test-tube. To illustrate better, C. N. Myers,[1] speaking of arsphenamine, states as follows:

"..... The cause of all chemical reactions is to be found in the fundamental laws of energy changes. Every energy change can be factored into two parts, an intensity factor and a capacity factor. These last two factors are causes of disturbance in the administration of arsphenamine. It is well known that, when two substances are brought together, there is a tendency to equalize

the difference in the intensity of the intrinsic energy producing the chemical change and on this basis the technique of administration must be carried out with the utmost care. A more homely illustration might be used in which a piece of sodium hydroxide is dropped in concentrated sulphuric acid with a violent reaction, whereas if dilute solutions of the same reagents are used, no perceptible change is noted, even though the equilibrium point is the same."

The same laws apply to the subcutaneous and intravenous administration of most drugs. Concentrated solutions are apt to produce local or systemic reactions. The injection of more dilute solutions even though the ultimate dose is the same allows a more gradual reaction.

Concentrated solutions may also be hypertonic which condition is sometimes responsible for local irritation.

Of course, for solutions intended for intravenous use, the right concentration is vastly more important. They are generally *more dilute* than solutions used subcutaneously. As an example we might quote an article by K. F. Maxcy[2] entitled "Limitations to the Use of Quinine Intravenously in the Treatment of Malaria," in which he says in part:

"All the precautions which are observed in giving a dose of salvarsan should be observed in giving quinine. A sterile filtered solution of the quinine salt (dihydrochloride is generally recommended) should be prepared and diluted so that 1 cc contains not more than $^1/_2$ grain of the drug."

In the administration of arsphenamine experience has shown that the proper concentration goes a great way in eliminating severe reactions. This drug is generally given in a dilution of 1 to 20 or 25 cc of distilled water, and it has been found that in this form the formation of precipitates with the proteins of the blood is almost eliminated.

Whenever possible all solutions injected subcutaneously and more so those given intravenously, should be isotonic. This can be readily done by determining the tonicity by cryoscopic or freezing-point method. The freezing point of normal human blood is $-0.526°$ C., therefore solutions with a lower freezing point are hypertonic and those with a higher freezing point than $-0.526°$ C. are hypotonic with respect to human blood. In cases where a hypertonic solution must be used to reduce volume, then that concentration should be used which clinical tests have shown to be least irritating or causes the least reaction when injected subcutaneously or intravenously.

HYDROGEN-ION CONCENTRATION AND BUFFERS.

The rôle of hydrogen-ion concentration and its influence on all biochemical substances has been brilliantly demonstrated by the work of Sörensen, Michaelis, Clark and Lubs, and others. Living cells, especially, are closely controlled in their functioning processes by a limited hydrogen-ion concentration. The application of the principles of hydrogen-ion concentration to the science of bacteriology within the past half dozen years has opened a new line of approach to the complex problem of bacterial metabolism. It has clarified many problems relating to the culture media, the action of acids and alkalis on colloidal hydration, and the effect of buffers on bacterial growth.

Clark and Lubs, in their splendid book on the subject, state as follows:

"Hydrogen-ion concentration influences the condition in solution of every substance with acidic or basic properties—native proteins and their hydrolytic products, amines and amides,

carboxyl, sulphonic, and phenolic compounds. It has a large effect on the effective solubilities and dispersion of colloids, upon determining tautomeric equilibria, and in one way or another in governing the activity of catalysts such as hydrolytic enzymes and oxidases. One or the other of these effects, induced directly or perhaps indirectly by the hydrogen-ion concentration, must impress bacterial life."

The hydrogen-ion concentration of sterile pharmaceutical solutions has received little or no attention. The few isolated cases which have appeared in literature on the subject seem to indicate that this factor can no longer be ignored or neglected. It is safe to state that the time is soon coming when the reaction of sterile pharmaceutical solutions will be determined in terms of hydrogen-ion concentration by colorimetric means or by the more accurate electrometric method, and that its influence on these solutions will be as marked as that in bacteriology.

Before discussing the importance of hydrogen-ion concentration on pharmaceutical solutions, it might be of advantage to explain very briefly acidity and alkalinity in terms of hydrogen-ion concentration, the meaning of buffers, and the rôle they play in maintaining the proper equilibrium in which hydrogen-ion concentration is concerned.

According to the dissociation theory of Arrhenius, when an acid of the hydrochloric acid type is dissolved in water, the acid splits or dissociates almost completely into positive hydrogen and negative chlorine ions. The acid properties of the acid and for that matter of all solutions are due to the hydrogen ions they contain. Some acids such as hydrochloric which are largely dissociated produce a high concentration of hydrogen ions and are classed as strong acids. Acids of the acetic acid type are only slightly dissociated, produce a low hydrogen-ion concentration, and are classed as weak acids. The strength of an acid, therefore, depends absolutely on the number of hydrogen ions present in a certain volume of a solution, that is, on the hydrogen-ion concentration and not on the amount of acid present. At this point it is necessary to emphasize the distinction between titratable acidity and hydrogen-ion concentration. The amount or quantity of acid present is determined by ordinary titrations whereas the degree or intensity of acidity is found by a hydrogen-ion determination made colorimetrically by means of indicators or electrometrically by using hydrogen and calomel electrodes properly connected to some form of a potentiometer. Similarly bases depend for their alkaline properties on the presence of hydroxyl ions, and the strength of a base is proportional to the number of hydroxyl ions present in its solutions. In other words the titrimetric method tells us the total quantity of acid hydrogen available in a given solution but does not tell us what fraction of the total acid hydrogen atoms present are dissociated into hydrogen ions, so that in one case we get the quantity factor and in another case the intensity factor of acidity.

Hydrogen-ion concentration is not expressed in terms of normality but by a symbol known as p_H or potential hydrogen and is equal to the logarithm of the reciprocal of the hydrogen-ion concentration or mathematically expressed $p_H = \log 1/[H^+]$. The p_H of pure conductivity water is 7. This is taken as the point of true neutrality, so that solutions having a p_H value above 7 are alkaline and those below 7 are acid.

The reaction expressed in p_H of a few well-known substances is as follows:

Tenth-normal hydrochloric acid	1
Gastric juice	0.9–1.6
Milk (cow)	6.6–6.8
Blood	7.4
Small intestinal contents	8.3
Tenth-normal ammonium hydroxide	11.0

Closely related to hydrogen-ion concentration is a class of substances called buffers. If 1 cc. of 0.01 N hydrochloric acid is added to one liter of distilled water p_H 7.0 the resulting solution would be about p_H 5.0. If, on the other hand, the same amount of acid is added to a solution of sodium phosphate or to a 1% peptone solution of p_H 7.0, the change of p_H is hardly noticeable. This is called buffer action. By buffer action then is meant the ability of a solution to resist change in p_H through the addition or loss of acid or alkali. Clark[3] has compared the determination of hydrogen-ion concentration to a thermometer and the action of buffer salts to a thermostat.

Salts of weak acids such as phosphates, carbonates, citrates, acetates, borates, etc., exert strong buffer action. Albumins, peptones, amino acids, and proteins in general are also good buffers. The hydrogen-ion concentration of the blood is regulated with remarkable efficiency by means of buffers, such as bicarbonates, phosphates, and proteins. Were it not for the presence of these buffers the slightest amount of acid or alkali injected into the blood stream would increase the acidity or alkalinity to such an extent as to produce symptoms of acidosis or alkalosis.

It is obvious, therefore, that the hydrogen-ion concentration and means of controlling it by buffers enter into consideration in all biochemical reactions. In the preparation of pharmaceutical solutions intended for subcutaneous or intravenous use one must bear constantly in mind the principles just discussed. Wherever possible, the solutions should be adjusted by means of buffer salts to the same reaction or p_H of the blood. This method produces solutions that are least irritating and more stable. In many cases the drug in solution undergoes hydrolysis or decomposition on sterilizing by heat or on standing a long time in the market containers. The result is a product that is physiologically or therapeutically inert. This hydrolysis can be prevented or retarded by controlling the hydrogen-ion concentration with buffer salts. A few examples will illustrate the point more clearly. Levy and Cullen[4] studied the relation of hydrogen-ion concentration to the deterioration of crystalline strophanthin in aqueous solution. They summarize their results thus "Many of the glass containers commonly used in the laboratory, and most of the glass ampules employed in marketing sterile solutions for hypodermic or intravenous medication, yield sufficient alkali on autoclaving to change the reaction of distilled water from p_H 6.0 to p_H 9.0.

"This increase in alkalinity is sufficient to render biologically inert and partially to decompose aqueous solutions of crystalline strophanthin in the concentration ordinarily employed in the clinic.

"It is suggested that for clinical use crystalline strophanthin be dissolved in 0.02 M standard phosphate solution at p_H 7.0 and marketed in hard glass ampuls, thereby insuring stability of reaction with preservation of biologic activity.

"It should be borne in mind both by laboratory worker and pharmacist that the alkali yielded, on heating, by soft glass containers may be responsible for a considerable alteration in the hydrogen-ion concentration of their contents."

Macht and Shohl[5] found that the stability of benzyl alcohol solutions is effected by slight amounts of alkali. They state "Various specimens of benzyl alcohol solutions were sealed in ampules made of different kinds of glass, and such solutions were examined at intervals of time in regard to their anesthetic efficiency on the one hand, and their hydrogen-ion concentration on the other.

"Solutions of benzyl alcohol kept in non-soluble glass preserve their anesthetic properties completely for long periods of time and such solutions tend to increase their hydrogen-ion concentration very slowly.

"Benzyl alcohol solutions kept in soft glass or alkaline containers tend to become alkaline in reaction and rapidly deteriorate in their anesthetic efficiency.

"It is suggested that the best method of preserving benzyl alcohol solutions is to prepare such solutions from a benzyl alcohol free from benzaldehyde and to seal the same after the addition of a buffer solution in hard glass ampules."

Rippel[6] found that solutions of certain alkaloids such as cocaine and its derivatives undergo hydrolysis in an alkaline medium resulting in the loss of activity.

While searching for the cause of the spontaneous deterioration of certain indicators, Mellon, Slagle, and Acree[7] were led to study the purity as measured by their p_H values. These observations revealed a high acid point for some of these dyes, particularly phenolsulphonphthalein, which is so extensively used in kidney efficiency tests. To quote the authors: "As we had been told previously by Dr. Williams of the apparent toxicity of certain batches of phenolsulphonphthalein in the marketed product, it occurred to us that there might be a correlation between p_H and the toxicity." They suggest methods for buffering solutions of phenolsulphonphthalein to prevent increase in toxicity in market containers.

Williams and Swett[8] have made a study of the hydrogen-ion concentration on distilled water, physiological sodium chloride, glucose, and other solutions used for intravenous medication. They bring out the fact that hydrogen-ion concentration of drugs and chemicals used for therapeutic purposes has never been given proper consideration. Clinicians have reported that the intravenous injection of glucose, physiologic sodium chloride, phenolsulphonphthalein, and other solutions is followed by reactions which seem to indicate that they are due to improper hydrogen-ion concentration. They also bring out the fact that in subcutaneous injections hydrogen-ion concentration is also of importance. An abnormal p_H may produce a localized temporary acidosis or alkalosis and that this may explain local reactions evidenced as "sore arms" commonly seen in hospital wards following hypodermic medication. They quote the results on the hydrogen-ion concentration of distilled water, glucose, and physiologic sodium chloride.

Many other instances could be given of the influence of hydrogen-ion concentration and the value of buffering solutions. The reader, however, is probably sufficiently impressed with the examples quoted to know that hydrogen-ion concentration does not only effect the vitality of living cells but also the stability and therapeutic efficiency of pharmaceutical solutions in market containers.

The application of these physico-chemical principles to the preparation of iron cacodylate, phenolsulphonphthalein, glucose and other solutions in the laboratory with which the writer is connected have given very encouraging results.

STERILIZATION.

Sterilization may be accomplished by heat or by filtration through diatomaceous or other suitable filters. Sterilization by heat may be brought about in several different ways—namely, by autoclaving under steam pressure, by heating at 100° C. or by fractional sterilization for three or four days at 50° to 60° C. The choice of method depends entirely upon the substances to be sterilized. Materials, such as caffeine, sodium cacodylate, pituitary extract, physiological salt, etc., which do not decompose at high temperatures may be sterilized in the autoclave for one hour at 115° C. In the case of solutions of substances which undergo decomposition at this temperature (adrenalin, atropine, morphine, cocaine, sparteine, etc.) sterilization may be accomplished by heating at 100° C. or by filtration. Substances which decompose at 100° C. (heroin, strophanthin, calcium glycerophosphate, etc.) may be either sterilized by intermittent sterilization or by candle-filtration. Some substances such as scopolamine, hyoscyamine, and organotherapeutic preparations can be sterilized with safety only by candle-filtration.

It is obvious that the method of sterilization is an important factor upon which depends the therapeutic value and stability of the preparations in the market containers. The greatest care must be exercised in the selection of the right method of sterilization.

To do efficient sterilization work a laboratory must be completely equipped with autoclaves and Arnold sterilizers controlled by thermostats and self-recording thermometers. Each individual filter-candle should be carefully tested for leaks before using.

Moreover, experience has shown that regardless of the method used in sterilization, one cannot take it for granted that the material is sterile without confirming it by cultural tests. A certain percentage of the market containers should be tested for sterility according to the method prescribed by the U. S. Hygienic Laboratory, thus leaving no doubt as to the sterility of the final product.

A word might be said about the selection of ampuls. Only insoluble glass should be used and each lot of ampules should be tested for solubility, thus insuring ampuls that will not give off alkali or glass spicules on long standing.

TOXICITY TESTS.

Experience has shown that ordinary chemical analysis does not reveal the presence of small amounts of toxic impurities, or of slight differences in the molecular structure of a compound. Two lots of a certain drug may analyze the same but yet differ considerably in toxicity. Therefore, certain drugs, notably organic arsenicals, must be tested biologically on suitable laboratory animals to determine their toxicity. Either white mice or white rats are used for this purpose.

Solutions of drugs that are intended for subcutaneous use are generally tested on white mice. In determining toxicity the U. S. Hygienic Laboratory Method is generally followed in which at least five mice are inoculated subcutaneously on each dose and at least 60% of the mice must survive 48 hours or more. On the other hand, solutions of drugs that are to be injected directly into the blood stream are tested for toxicity on white rats. The test solution is injected into the saphenous vein of the rat by means of a burette or a syringe accurately graduated to 0.01 cc. The U. S. Hygienic Laboratory states that for each toxicity test a series

of not less than five rats shall be used and at least 60% of the animals injected must survive at least 48 hours from the time of the injection. While these rules were applied to the testing of arsphenamine and neo-arsphenamine, the same methods may be used for determining the toxicity of other drugs.

The results obtained on these animal tests may be utilized in formulating the proper dose, concentration, and solvent to be used in preparation intended for human use.

CLINICAL TESTS.

It is sufficient to state, however, that in spite of careful physico-chemical and biological considerations, the real proof of a good, stable, potently therapeutic, comparatively non-irritating and non-toxic pharmaceutical preparation lies in the end results, and these are satisfactory clinical tests. Therefore, the work of a laboratory must be so planned as to coördinate the manufacturing, chemical, biological and clinical facts.

In conclusion, then, sterile pharmaceutical preparations can be elevated to a higher scientific place by the application of known physical and chemical principles to physiological processes.

MULFORD BIOLOGICAL LABORATORIES,
GLENOLDEN, PA.

REFERENCES.

1. C. N. Myers, "Development of the Chemotherapy of Organic Arsenicals and the Related Physical Phenomena," *J. Infectious Diseases*, 7, No. 1, 1921.

2. K. F. Maxcy, "Limitations to the Use of Quinine Intravenously in the Treatment of Malaria," *Public Health Reports*, 37, No. 12, 693.

3. W. M. Clark, "The Determination of Hydrogen Ions," Williams & Wilkins, Baltimore.

4. R. L. Levy and G. E. Cullen, "Deterioration of Crystalline Strophanthin in Aqueous Solution," *J. Expt. Med.*, 31, No. 3, 267, 1920.

5. D. I. Macht and A. T. Shohl, "The Stability of Benzyl Alcohol Solutions," *J. Pharm. and Expt. Therap.*, 16, No. 1, 61, 1920.

6. A. Rippel, "Influence of Their Reactions on the Permanence of Cocaine Solutions," *Arch. Pharm.*, No. 258, 287–95, 1920.

7. R. R. Mellon, E. A. Slagle and S. F. Acree, "The Practical Application of 'Buffers,'" *J. Am. Med. Assoc.*, 78, No. 14, 1027, 1922.

8. J. R. Williams and M. Swett, "Hydrogen Ion Concentration Studies," *Ibid.*, 78, No. 14, 1024, 1922.

ORGANOTHERAPEUTICS FROM THE PHARMACIST'S STANDPOINT.*

BY DAVID KLEIN, PH.D.

The administering of gland desiccations and derivatives is a comparatively new branch of therapeutics. Interest in this field, on the part of physicians, is increasing rapidly, with a resulting increase in the number and brands of products that are being offered to the practitioner and to the pharmacist. Concise information concerning the source and manufacture of the various glandular derivatives is not readily available; yet the intelligent purchasing, handling and dispensing of these products must be based upon such knowledge. It is with the hope of clear-

* An address without notes on the above subject was delivered before the Chicago Branch of the American Pharmaceutical Association, March 10, 1922. This paper follows closely the subject matter of that address, although a portion of it, centering around the gland specimens displayed at that time, must obviously be omitted.