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The Poisonous Effect Exerted on Living Plants by Phenols.

By

Rodney H. True, Ph. D., and Carl G. Hunkel, B. S.[1])

Introduction.

The interdependence of the sciences is abundantly illustrated by recent work bearing on the relation existing between the physico-chemical properties of solutions and their physiological action on living organisms. The application of the theory of the dissociation of electrolytes to explain the toxic action of acids,

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*) Für den Inhalt der Originalartikel sind die Herren Verfasser allein verantwortlich. Red.

[1]) Aug. Uihlein Fellow in Pharmaceutical Chemistry, University of Wisconsin.

bases and salts on living organisms has yielded results of greatest import to both chemistry and biology.

This relation was first demonstrated experimentally by a long series of experiments on plants by Kahlenberg and True.[1] These authors, though confining their experiments to a higher type of plants (*Lupinus albus* L.), recognized the wide range of phenomena for which this theory furnishes an explanation and pointed out from the data of other experimenters the adequacy of this theory to explain the action of antiseptics. At a later date, Kahlenberg and True[2]) presented in greater detail their results published previously in the preliminary article cited. At the same time, Heald, who had repeated the work of these authors with other test plants (*Pisum sativum*, *Zea mais* and *Cucurbita pepo*), published results confirming the conclusions obtained by these authors.[3]

Some three months later Paul and Kroenig,[4] following the same line of argument, showed by experiment that the extension of this theory to explain the action of antiseptics, as had been indicated by Kahlenberg and True, was amply justifiable.

The general principle being established for salts, acids and bases, it was believed by the writers that the extension of this method of study to that class of bodies known as phenols might lead to interesting results. This group of compounds lends itself also to the study of a further question, the specific influence of chemical radicles.

Methods.

Our problem has been to test on living plants the action during short periods of time exerted by phenols of various composition in solutions of varying degrees of concentration. In each case we have sought to establish the greatest concentration of each compound which will permit radicles of *Lupinus albus* immersed in it to live and make unmistakable growth. Lupines were germinated according to the usual method. From the stock of material at hand, plants of healthy appearance were selected and with India ink marked with lines 15 mm from the root tips, a distance sure to include the entire growing zone of the root. They were then fastened in a convenient manner in beakers containing the aqueous solutions of the desired substances at the desired concentrations. Care was taken, of course, to keep the cotyledons out of the solutions. Usually four radicles were used in each experiment.

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[1] Kahlenberg and True. On the toxic action of dissolved salts and their electrolytic dissociation. (Journ. Am. Med. Assoc. July 18, 1896.)

[2] Kahlenberg and True. On the toxic action of dissolved salts and their electrolytic dissociation. (Bot. Gaz. XXII., Aug. 1896. p. 81.)

[3] Heald, F. D. Toxic effect of acids and salts upon plants. (Bot. Gaz. XXII, Aug. 1896. p. 125.)

[4] Paul und Kroenig. Ueber das Verhalten der Bakterien zu chemischen Reagentien. (Zeitschr. f. physik. Chemie. XXI, Nov. 1896. p. 414.)

Since the question of the life or death of the radicles after an exposure to the various solutions is a point important to establish beyond doubt, great care was taken to insure certainly here. By a system of measurements, it was possible to determine the growth of the radicle during and after an exposure to the solution. Experiments showed that solutions of markedly harmful action either bring about the death of the radicles comparatively promptly or the radicle fights a long-continued, losing fight. Wishing to avoid decisions on the condition of radicles known to be abnormal, it seemed best to accept the condition seen after an arbitrary period had elapsed as the desired result. Accordingly, radicles were exposed from twenty to twenty-four hours to the action ot the solutions and their condition after the lapse of that time determined.

In determining whether at this time the radicles were alive or dead, several things were considered. The appearance of the roots as regards, color, turgidity, etc., was carefully noted. The growth made during the time spent in the solution was ascertained at the end ot the time of exposure by comparing the distance from the India ink line to the tip with fifteen millimeters, the invariable distance at the beginning of the experiment. Usually some growth was found to have taken place, only the stronger poisons causing death so soon as to show no elongation on the second measurement. In order to ascertain if further growth was possible, the radicles were returned to the solutions and after one or more days were again measured. If living at the end of the arbitrary period of exposure, some undoubted growth would appear. If none such was to be seen, and the superficial appearance, the condition relative to turgidity, etc., seemed to confirm the diagnosis, death was assumed. If the issue still remained uncertain, another return to the solution and another measurement followed until some result could with a fair degree of certainty be predicated. It was found to be useless to attempt to establish with accuracy the critical concentration, since variations due to the biological factors involved were hardly calculable.

Comparison experiments were also conducted in some cases with Spirogyra. The methods used are described later under the discussion of the action of phenol.

The solutions used were accurately prepared from trustworthy chemicals. A stock solution, made up on the basis of gram-molecules per liter, concentrated enough to cause prompt death to the radicles, was used as a starting point. A number of dilutions were then made, each of the series being half as concentrated as that next stronger, and the radicles were immediately placed in them. Thus all the experiments of a series were made contemporaneously in fresh solutions. Occasionally intermediate concentrations were interpolated when greater accuracy seemed likely to be attained. By using stock solutions made up at a strength equal to some power of one-half a gram-molecule per liter of water, we obtained chemical quantities standing in point of concentration in an easy mathematical ratio to each other and, therefore, readily comparable.

Experimental Results.

In general, the phenols were found to act with less sharpness and precision than acids and metals, and oftentimes some difficulty was experienced in determining the desired limit-concentration. Accordingly, a large part of the work was repeated one or more times. In order to compare more readily some of the physico-chemical and the toxic properties of the substances under study, the results obtained by Richard Bader,[1]) who made a study of the electrical conductivity of many phenols, accompany the biological data obtained by the authors.

In experimenting on the electrical conductivity of the phenols, Bader encountered a number of difficulties which he was unable to overcome. Phenol and some of its homologues, as also di- and trioxybenzenes, dissociate to but a very slight degree. The conductivity of the water used has to be determined and corrections made for the same in each instance. As it was imposible to foresee how the conductivity of water would affect the conductivity of the compound, the corrections were omitted in the tables of results. It was noticed that some of the solutions became brown in the measuring flask, owing to the oxidizing effect of the electrodes. The solutions were removed after each determination and replaced by the next dilution. By operating rapidly, decomposition was almost entirely obviated. Inasmuch as no sufficiently accurate method exists for the titration of these compounds, no other method could be pursued.

In the tabulated results given below, the following data are found. The date designated at the beginning of the experiment indicates the time at which the radicles, provided with the India ink line 15 mm from the tip were, placed in the solution named. In the left-hand column is indicated the concentration in fractions of a gram-molecule of the substance in question per liter of water. In the second column, the length of the radicles measured from the India ink line to the tip is given as found by measurement made at the date given at the head of the column, usually about twenty-four hours after the beginning of the experiment. The amount of growth made during this period is obtained by subtracting 15 from the lengths given in millimeters. In case column three is also headed with a date, the numbers given in the column indicate the lengths from tip to line found when measurements were made at the date indicated. The column headed „Condition“ contains the verdict rendered on each radicle after its exposure to the solution in question.

Although many trial experiments were made in locating the critical concentration, only those appear in the following tables which give decisive evidence.

Phenol, C_6H_5OH.

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[1]) Bader, R. (Zeitschr. f. physik. Chemie. VI. p. 289.)

Table I.

Begun March 22, 3 : 20 P. M.

Gram. mol. per liter.	Length Mar. 23, 3 : 40 P. M.	Length Mar. 25, 2 : 10 P. M.	Condition.
1/200	18,5 mm	19,0 mm	Dead.
	17,0 „	18,0 „	„
	17,5 „	17,5 „	„
	17,0 „	17,0 „	„
1/400	20,5 „	28,5 „	Alive.
	21,5 „	31,0 „	„
	22,5 „	28,0 „	„
	16,0 „	19,5 „	„

Table II.

Phenol + 1 NaOH. Begun Apr. 9, 5 : 10 P. M.

Gram mol. of phenol per liter.	Length Apr. 10 11 : 00 A. M.	Length Apr. 13, 4 : 00 P. M.	Condition.
1/200	16,5 mm	16,0 mm	Dead
	16,0 „	—	„
	19,0 „	20,0 „	„
	17,0 „	17,0 „	„
1/400	15,0 „	14,5 „	„
	17,0 „	24,0 „	Alive
	17,0 „	22,5 „	„
	17,5 „	29,5 „	„

Table III.

Electrical conductivity of Phenol.

V.	μv	100 k.
25	0,14	0,00000056
50	0,23	0,00000077
100	0,41	0,00000120

$\mu\infty = 357.$ K = ?

As appears in Table I., phenol ranks among the substances strongly harmful toward the life of the lupines, the first concentration in which these plants could survive being one gram-molecule in 400 liters. Experiments[1]) carried out with Spirogyra indicated that the alga tolerates this poison in larger quantities than the lupine. Small tufts of filaments were rinsed out in a solution of the strength to be used in the experiment to avoid a dilution of the same and placed in the desired concentration. After about twenty-four hours, their condition was examined and plasmolysis with a 5 per cent saltpeter solution was regarded as sufficient evidence of life in the cells. The limit-concentration for Spirogyra was found to be one gram-molecule in 200 liters. In this concentration, Bokorny[2]) found that this alga formed starch in five days. In a 0,1 per cent solution, Loew[3]) obser-

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[1]) True, R. H. Algae and antiseptics. (Pharm. Rev. XV. p. 152. 1897.)

[2]) Bokorny, Chem. Zeitung. 1894. No. 2. Cit. Biolog. Centralbl. XVII. p. 7. 1897.

[3]) Loew, Natürliches System der Giftwirkungen. p. 50.

ved the appearance of numerous, strongly refracting granules in cells of Spirogyra. Algae (not designated) are reported by Davenport[1]) to succumb in a 0,1 per cent solution after three days.

It seemed desirable to try to investigate more closely the action ot phenol, bearing in mind the possible formation of destructive ions. Accordingly, a series of experiments was made with solutions to which an equivalent amount of sodium hydroxide had been added. It is known that phenol unites with sodium and potassium hydroxide to form compounds, e. g., C_6H_5OK, in a manner similar to acids. How far this obtains in dilute solutions is problematical, but many reasons exist for supposing that hydrolytic dissociation goes on as rapidly as association. It is advisable to assume that in the solution of sodium hydroxide plus phenol there exist these two constituents. Kahlenberg and True find[2]) that lupines first grow in solutions of KOH when they are diluted to one gram-molecule in from 200 to 400 liters, and NaOH has probably the same limit. If we suppose that phenol does dissociate into $\overset{+}{H}$ ions and $C_6H_5\overset{-}{O}$ ions, the addition of an alkali would give a salt which would dissociate into $\overset{-}{C_6H_5O}$ ions and $\overset{+}{Na}$ ions. Since $\overset{+}{Na}$ ions have relatively a weak toxic effect,[3]) the difference between the limit-concentrations for phenol and for phenol plus KOH would be the measure of the action of the H ions. As far as the mixed solutions of phenols and the hydroxides of the alkali metals are concerned, it should be borne in mind that whenever the ratio of dissociation is greater for the metallic hydroxide than for the phenol, there will be $\overset{-}{OH}$ ions whose toxic action must not be overlooked. Tables I and II show however the same limit for both substances. Hence the toxic action must be due to the undissociated phenol. Kroenig and Paul[4]) arrive at the same conclusion from work done with bacteria. These authors find, however, that the sodium phenolate has less marked disinfecting properties than phenol. As will be seen in Table III, Bader strengthens our conclusion by his study of the electrical conductivity of the phenol solution, dissociation being found to be almost nil.

In view of Scheurlen's[5]) statement that the addition of NaCl intensifies the antiseptic action of phenol, it was thought desirable to test the matter with the lupines. Accordingly solutions containing phenol and NaCl in three different proportions were

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[1]) Davenport, Experimental morphologie. Part I. 1897. p. 18.

[2]) Kahlenberg and True. Bot. Gaz. XXII. (1896.) p. 95.

[3]) That the Na ion is entirely lacking in toxic action in strong solutions or in weaker solutions acting for a long time cannot be asserted.

[4]) Kroenig und Paul, Die chemischen Grundlagen der Lehre von der Giftwirkung und Desinfection. (Zeitschrift für Hygiene und Infections-Krankheiten. Band XXV. 1897. p. 84.)

[5]) Scheurlen, Die Bedeutung des Molecularzustandes der wassergelösten Desinfectionsmittel für ihren Wirkungswerth. Strassburg. 1895.

used: 1. one gram-molecule of phenol per gram-molecule of NaCl, 2. one gram-molecule of phenol to two gram-molecules of NaCl, and 3. one gram-molecule of phenol to three gram-molecules of NaCl. The results obtained failed to show this action in any distinct manner. The concentration-limits marking the survival of the lupines as calculated on the basis of the phenol present were 1/400 gram-molecule for the first and second solutions and somewhere between 1/400 and 1/800 gram-molecule per liter for the third solution. How much NaCl must be added in order to increase materially the toxic action of phenol toward the lupines was not farther investigated. Krönig and Paul were able to observe this increase in working with bacteria; in this case, however, very much stronger solutions of all kinds were used, and it is not impossible that the concentration of the solution may in some way influence this action.

Of the di-atomic phenols the isomers, pyrocatechol, resorcinol and hydroquinone, were studied.

Pyrocatechol, C_6H_4 (ON) (OH) = 1 : 2.

Table IV.

Begun April 28, 7 : 40 P. M.

Gram mol. per liter	Length Apr. 29, 3 : 45 P. M.	Length May 1, 2 : 20 P. M.	Condition.
1/400	17,0 mm	18,0 mm	Dead.
	19,5 „	22,0 „	„
	20,0 „	22,0 „	„
	19,0 „	18,5 „	„
1/800	19,5 „	26,0 „	Alive.
	20,0 „	24,0 „	„
	15,0 „	15,0 „	Dead.
	17.0 „	20,0 „	Alive.

Table V.

Electrical conductivity of Pyrocatechol (Bader).

V.	μv	100 k.
16,4	0,13	0,00000079
32,8	0,33	0,0000026
65,6	0.61	0,0000044

$\mu\infty = 356.$ K = ?

(To be continued.)

Leuchten unsere Süsswasserperidinien?

Von

Prof. Dr. **F. Ludwig.**

In einem Aufsatz „die Welt im Wassertropfen“, in Unterhaltungen aus dem Gebiet der Natur, herausgegeben vom österreichischen Lloyd in Triest, 1856, berichtet Ferdinand Cohn auf p. 43 folgendermassen über ein leuchtendes Süsswasser

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