THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE TOXICITY OF NICOTINE, PYRIDINE, AND METHYL-PYRRROLIDINE TO MOSQUITO LARVAE

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INTRODUCTION

Previous investigations of the toxicity to insects of nicotine in aqueous solution have involved principally a study of the effects of its application as a spray or dip. The solutions employed have generally contained nicotine sulphate and soap or other alkaline substance, the purpose of the latter being to increase the wetting and spreading properties of the liquid and to convert the nicotine salt into the uncombined, volatile base. After the application, the insect is in contact with continuously varying quantities of nicotine in solution and nicotine gas as evaporation of the alkaloid and water progresses. Under these conditions the toxic effect of nicotine has been explained as the result of its entrance in the gaseous state through the spiracles into the tracheal system of the insect.

In addition to the variable concentration, the results of treating insects by the method just mentioned are complicated by the presence of the alkaloid in three conditions, viz, as gas molecules, as molecules in solution, and as ions. It seemed probable that additional light could be thrown upon the nature of the effect of nicotine upon insects if gaseous nicotine could be eliminated, leaving only the molecules and ions in solution to act upon them. Then, by varying the hydrogen-ion concentration, the insects could be subjected to solutions differing widely in relative content of dissolved molecules and ions.

Accordingly, an aquatic insect, the larva of the house mosquito (Culex pipiens L.) was chosen for the purpose and proved to be an excellent test insect.

EXPERIMENTAL PROCEDURE

MATERIALS AND METHODS

The mosquito larvae were reared in a greenhouse in battery jars 15 cm. in diameter. Each of the jars contained approximately 3 liters of tap water in which a quantity of soil and decayed leaves was suspended. Within 24 hours most of the solid matter fell to the bottom of the jars, leaving a solution rich in food for the larvae. Female mosquitoes entered the greenhouse on warm nights and oviposited freely on the surface of the water in the jars. Each morning the egg boats were collected and distributed among the jars so that each would contain the desired number of larvae of about the same age. The experiments extended from July 1 to November 1, 1927.

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All nicotine solutions used were made up to the desired molar concentration by diluting 96 per cent nicotine with distilled water. The solutions of nicotine sulphate and nicotine hydrochloride were adjusted to the desired hydrogen-ion concentration with 0.1 normal sulphuric or 0.1 normal hydrochloric acid solutions, respectively, and then diluted. The pH values were determined colorimetrically and checked by the hydrogen electrode, using a saturated calomel half cell, a saturated potassium chloride salt bridge, and a Clark shaking hydrogen electrode assembly with platinum electrodes. The values obtained by the two methods agreed within about 0.1 pH, the electrometric values always being higher.

A group of 10 active, feeding larvae in the last instar was used for each experiment except the tests with 0.1 molar solutions in which the time of action was very short. The larvae were removed from the culture to a small dry beaker by means of a medicine dropper with a wide orifice. The culture solution carried over with the larvae was then removed until not more than about 0.1 c. c. remained in the beaker. Thirty-five cubic centimeters of the nicotine solution at the temperature of the water bath were gently poured into the beaker, which was then placed in a water bath maintained at 26±1° C. A few experiments were made at slightly lower temperatures, but the limits for the entire series fell between 24° and 27°. The volume of the solution stated above was not rigidly adhered to, and sometimes was as low as 12 c. c. Since 10 larvae of the size used in these experiments weigh about 32 mgm., the quantity of nicotine solution was always very large as compared with the mass of the larvae.

The time to the nearest second from the immersion of the larvae in the nicotine solution to the time the fifth one of the 10 became immobile was taken as the time of action of the particular nicotine concentration for 50 per cent of the larvae in each experiment. The action of 0.1 molar nicotine solution was so rapid that it was necessary to reduce the number of larvae in each experiment to four.

THE PROCESS OF POISONING

The course of poisoning of Culex larvae in a nicotine solution requires some consideration at this point. In a 0.03 molar solution the larvae swim about for a brief period, making few excursions to the surface. Soon, however, they fall writhing to the bottom of the beaker, and at times make short, vigorous, uncoordinated swimming movements. Movement gradually subsides, and the body then stiffens into a state of comparative immobility. This was chosen as the end point, and it is quite decisive to an experienced worker. Slight twitching, especially of the siphon, still occurs in response to gentle pressure with a needle, the dorsal heart continues to pulsate, and movements of the digestive tract are visible through the translucent body wall.

The effects of other concentrations differ essentially from the one just described only in the time of appearance of the characteristic phenomena. Life continues for a long, but variable, period following the beginning of this immobile stage. It is manifested by feeble body movement, irregular heart action, and movement of the digestive tract. Preliminary experiments have shown a certain degree
of recovery following transference to water after the first immobile stage is reached, but a quantitative study of it has not yet been completed.

Theoretically a compound like nicotine may enter the body of a submerged Culex larva by at least four routes: (1) Through the siphon and thence into the tracheal system; (2) through the tracheal gills on the anal segment into the tracheal system; (3) through the cuticula into the tissues and blood; (4) through either the mouth or anus into the digestive tract.

The siphon opening of the Culex larva is guarded by five petallike valves which are usually held tightly together when the larva is submerged but which open when the tip of the siphon is pressed against the surface film as the larva seeks a fresh supply of oxygen. Although Wesenberg-Lund observed that the valves are not always completely closed when the normal larva is submerged in water, frequent observations made by the writers showed that it was closed when the larva was placed in the nicotine solution. It is therefore believed that little if any nicotine, either as gas or in solution, enters the body through the siphon.

Structurally, the tracheal gills appear to offer an excellent opportunity for the passage of substances dissolved in water. However, no evidence that the gills are permeable to nicotine was obtained from this study.

The chitinous cuticula of the larva is probably at most slowly permeable to nicotine. Indirect evidence of its impermeability to this compound is furnished by experiments with Culex pupae, which survive for long periods (20 hours or more) in nicotine solutions (0.003 M and 0.012 M) and may even transform to adults before death. The pupae have no mouth opening. In view of the lack of evidence to the contrary, the writers believe that the toxic effects of nicotine in solution produced in Culex larvae are not due to the passage of the compound through the chitinous cuticula.

When first placed in a weak solution of nicotine the larva swims about, making typical feeding movements with the rotary mouth brushes. As soon as the effects of the poison become apparent, the larva opens the mandibles widely. Under these conditions it appears certain that the nicotine enters the digestive tract in quantities sufficient to produce toxic effects. Entrance through the anus is also a possibility, but evidence in support of it is lacking.

From a consideration of the structure of the larvae and their behavior in the solutions, it is probable that nicotine enters the body chiefly through the mouth as molecules or ions in solution rather than as molecules in the gaseous condition. Bodine concluded that certain acids and mercuric chloride penetrate mosquito larvae orally rather than cutaneously.

**EXPERIMENTAL RESULTS**

**THE TOXICITY OF NICOTINE AND NICOTINE SULPHATE SOLUTIONS AT VARIOUS pH VALUES**

Nicotine solutions of 0.03 molar concentration, titrated with 0.1 normal sulphuric acid solution to pH values from 2.4 to 7.0, were used in a series of experiments on mosquito larvae. The results are

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3 Reference is made by number (italic) to Literature Cited, p. 347.
summarized in Table 1, and are plotted in Figure 1 as reciprocals of the time in seconds to immobility of 50 per cent of the larvae. The toxic effect of the solutions changes at first slowly with rising pH value, then more abruptly, attaining a maximum for the solution of highest pH value, i.e., nicotine base. Similar results were obtained with solutions titrated to the desired pH with hydrochloric acid solution (Table 1), indicating that the toxicity of the various solutions was not due to a specific action of the sulphate ion.

**Table 1.—Toxicity of nicotine, nicotine sulphate, and nicotine hydrochloride solutions at various pH values to the larvae of Culex pipiens in 30 groups of 10 larvae for each test, 1927**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molar concentration of nicotine</th>
<th>pH</th>
<th>Dates of experiments</th>
<th>Mean seconds to immobility of 50 per cent of larvae</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine sulphate</td>
<td>0.03</td>
<td>2.4</td>
<td>Sept. 16, 20</td>
<td>2,425±65</td>
<td>14.7</td>
</tr>
<tr>
<td>Do</td>
<td>0.03</td>
<td>3.6</td>
<td>Sept. 17, 20</td>
<td>1,122±78</td>
<td>20.1</td>
</tr>
<tr>
<td>Do</td>
<td>0.03</td>
<td>5.0</td>
<td>Sept. 14, 20</td>
<td>1,304±43</td>
<td>17.3</td>
</tr>
<tr>
<td>Do</td>
<td>0.03</td>
<td>7.0</td>
<td>Aug. 16, 17; Sept. 21</td>
<td>307±37</td>
<td>39.6</td>
</tr>
<tr>
<td>Nicotine</td>
<td>0.03</td>
<td>9.7</td>
<td>Sept. 13, 15, 21</td>
<td>321±15</td>
<td>24.9</td>
</tr>
<tr>
<td>Nicotine hydrochloride</td>
<td>0.03</td>
<td>3.6</td>
<td>Oct. 27, 28, 29</td>
<td>1,882±77</td>
<td>22.4</td>
</tr>
<tr>
<td>Do</td>
<td>0.03</td>
<td>5.0</td>
<td>Oct. 4, 5, 28</td>
<td>1,363±49</td>
<td>19.5</td>
</tr>
</tbody>
</table>

* The values for the error of the mean ($\sigma_M$) were computed from the formula: $\sigma_M = \frac{\sigma}{\sqrt{N}}$, in which $\sigma$ is the standard deviation and $N$ the number of groups of larvae.

* The coefficient of variation is the ratio ($\sigma$ : mean seconds to immobility) multiplied by 100.

The curves for toxicity of nicotine to mosquito larvae and for the dissociation of nicotine are compared in Figure 1. The dissociation constants for a 0.01 molar solution at $15^\circ$ C. and the pH values of the neutral and basic salts of nicotine are taken from Kolthoff (9, 10). Nicotine is a weak diacid base. The first dissociation constant is $7.07 \times 10^{-7}$ (pK$_1 = 6.16$); the second dissociation constant is $1.12 \times 10^{-11}$ (pK$_2 = 10.96$). The first dissociation apparently involves the nitrogen of the pyrrolidine ring, the basic salt having a pH value of 5.6 whereas the second dissociation involves the nitrogen of the pyridine ring, the pH value of the neutral salt being 2.6. The pK value for pyridine, as given by Kolthoff, is 8.90.

An inspection of Figure 1 shows a close relationship between the first dissociation curve for nicotine and the curve for toxicity—both the percentage of dissociation and the speed of toxic action, expressed in reciprocals of the time in seconds elapsing until the larvae become immobile, being plotted against pH. The second dissociation, however, affects the shape of the toxicity curve somewhat, although to a very much less extent than does the first dissociation. As a result, in the region of the pH value of the basic salt (pH 5.6) toxicity approaches its minimum value.

These curves are believed to demonstrate two propositions: (1) That the speed of entrance of nicotine from an aqueous solution into Culex larvae is related to the concentration of undissociated nicotine base in the solution; (2) that in so far as the toxicity is concerned with the ionization of the nicotine molecules, it is governed largely by the dissociation of the pyrrolidine nitrogen. The latter statement is made on the assumption that the first dissociation of nicotine
must be governed by the pyrrolidine nitrogen in the same way that the second dissociation is governed by the dissociation of the pyridine nitrogen. The pK value of methylpyrrolidine has apparently not been determined but will probably be found to be less than 6.

THE TOXICITY OF NICOTINE AND NICOTINE SULPHATE SOLUTIONS AT DIFFERENT CONCENTRATIONS OF NICOTINE

Nicotine solutions of 0.1, 0.03, 0.01, and 0.001 molar concentrations were each titrated to pH 5.0 with 0.1 N sulphuric acid solution and compared in toxicity with solutions of the base at the same molar concentrations. The results are given in Table 2 and Figure 2.

![Figure 1](image_url)

**Figure 1.**—Toxicity to the larvae of Culex pipiens of 0.03 M nicotine at various hydrogen-ion concentrations compared with the dissociation of nicotine. The toxicity is plotted in terms of the reciprocals of the time $T$, to the seconds needed to produce immobility in the larvae. The dissociation curves are taken from the works of I. M. Kolthoff (9, 10). This figure shows the two series of data by superimposed graphs. The figures for the toxicity curve are shown at the left of the chart and the percentages for the dissociation curves at the right.

The speed of toxic action of the nicotine base, expressed as the reciprocal of the time in seconds to immobility, follows nearly a straight line between the concentrations of 0.01 and 0.1 molar. In this range the proportion of nicotine ions falls from about 3 to less than 1 per cent. The increase in toxicity with increasing concentration is therefore chiefly due to the effect of the content of undisassociated nicotine hydroxide molecules rather than to the nicotine-ion content. In the solutions containing nicotine sulphate (pH 5.0), more than 99 per cent of the molecules are in the ionic state, and the speed of toxic action for them at every concentration is much less than for the solutions of the base having the same nicotine concentration. When the ratio (seconds to immobility in the nicotine
# Table 2.—Toxicity of nicotine and nicotine sulphate solutions to the larvae of Culex pipiens at various base concentrations

[Nicotine sulphate solutions adjusted to pH 5.0]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molar concentration of nicotine</th>
<th>pH</th>
<th>Dates of experiments</th>
<th>Num. of larvae in each group</th>
<th>Num. of groups</th>
<th>Mean seconds to immobilization of 50 per cent of larvae</th>
<th>Coefficient of variation a</th>
<th>Mean ratio of toxicities of base and salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotine</td>
<td>0.1</td>
<td>9.95</td>
<td>Oct. 8, 10, 11, 21, 24, 26.</td>
<td>4</td>
<td>49</td>
<td>43.6 ± 1.8</td>
<td>29.3</td>
<td>6.7</td>
</tr>
<tr>
<td>Nicotine sulphate</td>
<td>0.1</td>
<td>5.0</td>
<td>Oct. 5, 6, 7, 21, 24, 26.</td>
<td>4</td>
<td>49</td>
<td>281 ± 14</td>
<td>35.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Nicotine</td>
<td>0.03</td>
<td>7.7</td>
<td>Sept. 15, 15, 21.</td>
<td>10</td>
<td>30</td>
<td>321 ± 15</td>
<td>24.9</td>
<td>6.8</td>
</tr>
<tr>
<td>Nicotine sulphate</td>
<td>0.03</td>
<td>5.0</td>
<td>Sept. 14, 20.</td>
<td>10</td>
<td>30</td>
<td>1,350 ± 43</td>
<td>17.3</td>
<td>6.8</td>
</tr>
<tr>
<td>Nicotine</td>
<td>0.03</td>
<td>9.7</td>
<td>July 20, 21, 22, 23, 24, 27, 28, 29; Aug. 2.</td>
<td>10</td>
<td>200</td>
<td>176 ± 5</td>
<td>43.8</td>
<td>6.8</td>
</tr>
<tr>
<td>Nicotine sulphate</td>
<td>0.03</td>
<td>5.0</td>
<td>July 23, 25, 26, 27, 28, 29; Aug. 1, 2.</td>
<td>1</td>
<td>200</td>
<td>992 ± 51</td>
<td>72.9</td>
<td>6.6</td>
</tr>
<tr>
<td>Nicotine</td>
<td>0.01</td>
<td>9.6</td>
<td>Sept. 7, 8, 9, 10.</td>
<td>10</td>
<td>20</td>
<td>1,622 ± 54</td>
<td>14.9</td>
<td>6.2</td>
</tr>
<tr>
<td>Nicotine sulphate</td>
<td>0.01</td>
<td>5.0</td>
<td>Sept. 9, 10.</td>
<td>10</td>
<td>20</td>
<td>9,783 ± 142</td>
<td>6.5</td>
<td>6.2</td>
</tr>
<tr>
<td>Nicotine</td>
<td>0.001</td>
<td>8.5</td>
<td>Sept. 1, 6.</td>
<td>10</td>
<td>20</td>
<td>4,056 ± 309</td>
<td>34.1</td>
<td>6.2</td>
</tr>
<tr>
<td>Nicotine sulphate</td>
<td>0.001</td>
<td>5.0</td>
<td>Sept. 7, 8.</td>
<td>10</td>
<td>20</td>
<td>^b 25, 200</td>
<td>^b 6.2</td>
<td></td>
</tr>
</tbody>
</table>

*The coefficient of variation is the ratio (σ: mean seconds to immobility) multiplied by 100.

b Estimated.

The toxicity of nicotine sulphate to seconds to immobility in the base is calculated for each group of larvae—the groups in each series of tests being arranged in pairs in chronological order of the experiments—the mean of all of the ratios tends toward values between 5 and 7; i.e., nicotine base at the concentrations used was about 5 to 7 times more toxic than nicotine sulphate at pH 5.0. This result was obtained with individual larvae as well as with groups of 10 larvae. (Table 2.)

THE EFFECT OF SUPPRESSING THE IONIZATION OF NICOTINE BY THE ADDITION OF HYDROXYL IONS

Since an increase in the concentration of unionized nicotine increases the toxicity of nicotine solutions, the question arises if toxicity can also be increased when the ionization of the nicotine base is suppressed by the addition of hydroxyl ions. However, the nicotine base is so slightly ionized that suppression of the ionization would be expected to yield very little additional toxicity. For instance, a solution containing 0.03100 mols of the nicotine base per liter theoretically contains 0.03085 mols of unionized nicotine per liter, solutions of 0.01000 molar strength contain 0.00992 mols of unionized nicotine per liter, and those of 0.001000 molar strength contain 0.000974 mols of unionized nicotine per liter. When these solutions are made to contain 0.01 mols of sodium hydroxide per liter the ionization of nicotine may be said to be completely suppressed, since in any of these
solutions there was less than 1 part in a thousand of nicotine that remained ionized. The suppression of ionization by the sodium hydroxide, therefore, only affected 4.7 parts in a thousand in the case of 0.031 molar nicotine, 8.4 parts in that of 0.01 molar nicotine, and 26 parts in that of 0.001 molar nicotine.

Solutions of 0.001 M, 0.01 M, and 0.03 M nicotine were made up to contain from 0.001 M to 0.05 M sodium hydroxide, the excess of hydroxyl ions being sufficient to suppress almost completely the ionization of the nicotine. The results are expressed in Figure 3 as ratios of the time in seconds needed to bring about immobility in nicotine solution containing sodium hydroxide to time in seconds to the same effect in nicotine alone.

Solutions of sodium hydroxide of a concentration of 0.05 M or less are comparatively nontoxic to Culex larvae, the larvae dying only after exposure to such solutions for from 2 to 12 hours.

The results with 0.03 M and 0.001 M nicotine show clearly that a further suppression of ionization of the nicotine leads to no significant change in toxicity, the ratio, 1.0, being closely approached in both cases. The values obtained for 0.01 M nicotine are, with the one exception shown, more irregular.

A series of experiments in which barium hydroxide, calcium hydroxide, and potassium hydroxide were substituted for sodium hydroxide gave toxicity values in good agreement with those for sodium hydroxide.

The conclusion reached may be stated as follows: In aqueous nicotine solutions of 0.001 M concentration or higher, the addition of an inorganic base increases the concentration of unionized nicotine so slightly that under these conditions no increase in toxicity can be observed.
THE TOXICITY OF PYRIDINE AND METHYLPYRROLIDINE

Pyridine solutions of 0.03 and 0.12 molar concentrations were titrated to pH 5.0 and 4.9, respectively, with 0.1 normal sulphuric acid, and their toxicities to Culex larvae were studied in comparison with solutions of pyridine base of the same concentration. A few experiments were also made with a small sample of methylpyrrolidine, the concentrations being 0.03 M, the pH values for the base approximately 9.8, and for the salt (hydrochloride) 3.0.

The results are given in Table 3. They show clearly that these compounds, components of the nicotine molecule, are more toxic as bases than as salts. Both compounds, either as bases or salts, are strikingly less toxic than nicotine, indicating that the toxicity of nicotine results from something inherent in the combination of pyridine with methylpyrrolidine rather than in either of its components.

**Table 3—Toxicity of pyridine and methylpyrrolidine (base and salt) to the larvae of Culex pipiens**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molar concentration</th>
<th>pH</th>
<th>Dates of experiments</th>
<th>Number of larvae in each group</th>
<th>Number of groups</th>
<th>Mean seconds to immobility of 50 per cent of larvae</th>
<th>Coefficient of variation of toxicities of base and salt</th>
<th>Mean ratio of toxicities of base and salt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyridine</td>
<td>0.12</td>
<td>7.8</td>
<td>Aug. 10, 11</td>
<td>1</td>
<td>40</td>
<td>702±26</td>
<td>25.3</td>
<td>2.2</td>
</tr>
<tr>
<td>Pyridine sulphate</td>
<td>0.12</td>
<td>4.9</td>
<td>Aug. 12</td>
<td>1</td>
<td>40</td>
<td>1,566±56</td>
<td>22.6</td>
<td>2.2</td>
</tr>
<tr>
<td>Pyridine</td>
<td>0.03</td>
<td>7.1</td>
<td>Nov. 1</td>
<td>10</td>
<td>10</td>
<td>2,048±81</td>
<td>12.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Pyridine sulphate</td>
<td>0.03</td>
<td>5.0</td>
<td>Nov. 3</td>
<td>10</td>
<td>10</td>
<td>4,432±34</td>
<td>2.4</td>
<td>2.2</td>
</tr>
<tr>
<td>Methylpyrrolidine</td>
<td>0.03</td>
<td>9.8</td>
<td>Nov. 4</td>
<td>10</td>
<td>4</td>
<td>2,895±106</td>
<td>7.3</td>
<td>2.2</td>
</tr>
<tr>
<td>Methylpyrrolidine hydrochloride</td>
<td>0.03</td>
<td>3.0</td>
<td>Nov. 4</td>
<td>10</td>
<td>6</td>
<td>54,000</td>
<td>18.6</td>
<td>2.2</td>
</tr>
</tbody>
</table>

*The coefficient of variation is the ratio (σ: mean seconds to immobility) multiplied by 100.

**DISCUSSION**

The idea that nicotine is more effective against insects than its salts seems to be an old one. In 1900, Del Guercio (6) reported some experiments in which silkworms (Bombyx mori L.) were sprayed with aqueous solutions of nicotine, nicotine containing alkali, and nicotine containing acid. No differences were noticed in the effects produced by the solution of nicotine and the nicotine containing alkali, but the acidulated nicotine was less active than the other solutions. Vermorel and Dantony (23, p. 23) added sodium carbonate to spray solutions containing a nicotine salt, explaining that its purpose was to liberate the nicotine from the combination. They further stated that it has been established for a long time that free nicotine is more toxic than nicotine salts. Holister (7) submerged bedbugs (Cimex lectularius L.) in solutions of nicotine and nicotine sulphate of various concentrations, and claimed a slightly higher toxicity for the nicotine solutions. McIndoo (15) published the first extensive work on the action of nicotine as an insecticide. He concluded that nicotine spray solutions neither pass into the tracheae nor penetrate the integument, but that nicotine vapor enters the body through the tracheae and passes thence to the tissues. He employed solutions of nicotine and of nicotine sulphate but reported

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*The sample of methylpyrrolidine was kindly furnished by F. B. LaForge of the Insecticide Division, Bureau of Chemistry and Soils.*
no difference in their action on insects. Moore and Graham (17) compared the effect of a solution of nicotine sulphate with a nicotine sulphate solution rendered alkaline with sodium carbonate upon the aphid *Macrosiphum sanborni* Gillette, the solutions being applied as sprays. The nicotine solution containing sodium carbonate was considerably more active than the nicotine sulphate solution without this addition. The results were held to be due to the presence of nicotine in volatile form in the alkaline solution. In a later paper (18) the same writers, confirming McIndoo's results, showed that nicotine solution does not penetrate into the tracheae but that nicotine vapor does enter them. De Ong (4, 5) sprayed and fumigated aphids with 0.1 per cent solutions of nicotine sulphate of various pH values from 6.5 to 8.2. The results from the two methods of application were parallel, the toxicity increasing with the increase of the pH value of the solutions. He believes that nicotine is a tracheal rather than a true contact insecticide. Worthley (25) has recently confirmed previous work in showing that alkalies increase the toxicity of nicotine sulphate solutions.

A few references to the relative toxic effect of nicotine as a base and as a salt on other animals than insects have been found in the literature. In 1889, Langley and Dickinson (12, p. 426) noted that if a nicotine solution were neutralized with sulphuric acid solution its effect both upon nerve fibers and upon ganglion cells was lessened. No explanation of these observations was attempted. Moore and Row (16) studied the comparative physiological action of nicotine, piperidine, and coniine upon the frog. They found that the free bases were more active than the respective salts, 2 mgm. of basic nicotine being as active as 10 mgm. of nicotine hydrochloride. De Ong (5) showed that free nicotine, administered orally, was very much more toxic to chickens than nicotine sulphate.

Studies on the toxicity of other alkaloids and similar compounds in relation to the reaction of the dissolving medium have been numerous, dating back at least as far as 1897, when Overton (19, p. 208) showed that ammonia, various amines, and many alkaloids enter cells readily as free bases, whereas the salts of these compounds if dissociated do not penetrate cells to a noticeable degree. Crane (3) found that the toxicity of alkaloids to *Paramecium caudatum* varied with the dissociation constant of the base and stated that the free, undissociated base is apparently responsible for the toxicity. No evidence of a direct action of the hydrogen ions upon the resistance of the cell was obtained. Labes (11) studied the effect of hydrogen-ion concentration upon the toxicity of certain alkaloids and acids to bacteria and frog larvae and concluded that the undissociated, lipid-soluble base was more toxic than the lipid-insoluble ions. Copeland and Notton (2) found that the action of a local anaesthetic depends upon the specific selective affinity of its base for nerve fibrils and that the different salts of these compounds vary in their action according to the degree of dissociation. Anaesthetic action is relatively high when the base is combined with a weak acid, the solution having a high pH value, and is relatively low when it is combined with a strong acid, the pH value then being lower. Trevan and Boock (22) have studied the relation of hydrogen-ion concentration to the action of a number of local anaesthetics on the rabbit's cornea. The results are held to be consistent with the view that the free base is the only
active constituent of the solutions, the ions or undissociated salt molecules taking no part.

The investigations of the writers referred to above indicate, in general, that nicotine base is more toxic than nicotine salt when administered to insects as a spray or dip, and that it is more toxic to vertebrates as the free base when administered either by mouth or by injection. The results of studies on other alkaloids and organic bases, in which a variety of organisms was used, are in accord with those on nicotine.

The greater insecticidal effect of nicotine as compared with nicotine sulphate has been attributed to the action of nicotine gas molecules which are able to enter and diffuse through the tracheal system. The present studies show, however, that the free base in aqueous solution may also be more toxic to insects than the salt.

It should be noted that nicotine solutions of pH 2.4 still possess an appreciable toxicity although less than that of the free base. This apparently signifies that the ions are, in themselves, somewhat toxic, for in solutions having the concentrations of those used in this study ionization of the nicotine salt molecules is nearly complete.

Although the penetration of gases into living cells is a familiar phenomenon (cf. Jacobs, 8, p. 133), it seems doubtful whether nicotine can long remain in this state within the tracheal system of an insect because of its great affinity for water. It therefore probably enters the cell as molecules or ions in solution. Lillie (13, 14), working with certain organic acids which produce their maximum effect as undisassociated molecules, holds that the acid molecules penetrate cell membranes rapidly and dissociate within the cell, yielding acid ions. Taylor (21, p. 218), who also worked with acids, believes that the hydrogen ions of the acid are adsorbed into the cell membrane, and the electric charge on the membrane then attracts the acid anion, the pair of ions gradually passing into the cell. It is not impossible that nicotine reacts with certain constituents of cells. The recent work of Petrunkin and Petrunkin (20, p. 108), in which they express the belief that certain alkaloids and organic bases combine with gelatin and brain proteins only on the alkaline side of the isoelectric point, are of interest in this connection.

**SUMMARY**

The toxicity of nicotine to the house mosquito (*Culex pipiens* L.) was studied in aqueous solutions at various pH values. A few similar experiments were also made with pyridine and methylpyrrolidine.

Solutions of 0.03 M concentration adjusted to pH values of 2.4, 3.6, 5.0, and 7.0, with sulphuric acid and at pH 9.7 (the free base) showed a toxic action that increased with increasing pH value. Solutions adjusted to pH 3.6 and 5.0 with hydrochloric acid gave similar results to those adjusted with sulphuric acid.

Solutions of 0.1 M, 0.03 M, 0.01 M, and 0.001 M concentrations were adjusted to pH 5.0 with sulphuric acid and compared in toxicity with solutions of the base of the same molar concentrations. At each nicotine concentration, the free base is about 5 to 7 times more toxic than is nicotine sulphate solution at pH 5.0.

The addition of an inorganic hydroxide (0.05 to 0.001 N) to an aqueous solution of nicotine is without apparent effect upon the toxicity of that solution.
Pyridine solutions of 0.03 M and 0.12 M were about twice as toxic as solutions of the same molar concentration titrated to pH 5.0 and 4.9, respectively, with sulphuric acid. Methylpyrrolidine solution (0.03 M) was about 19 times more toxic than methylpyrrolidine hydrochloride solution of pH 3.0.

The speed of toxic action to the larva of *Culex pipiens* of nicotine, pyridine, and methylpyrrolidine in aqueous solution is directly related to the concentration of the undissociated molecules.

It is believed that toxicity results largely from the penetration of the molecules into the body through the wall of the alimentary tract. Nicotine ions are somewhat toxic but much less so than nicotine molecules. It is also believed that the change in toxicity of a nicotine solution with change in pH results largely from the dissociation of the pyrrolidine nitrogen.

Previous writers have explained the greater toxicity of nicotine over nicotine sulphate on the basis of the greater volatility of the former. In this study it is shown that the free base in solution is also much more toxic than nicotine sulphate.

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