1927

Studies on the toxicity of hydrogen cyanide

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STUDIES ON THE TOXICITY OF HYDROGEN CYANIDE

by

James B. Allison

A Thesis submitted to the Graduate Faculty
for the degree of

DOCTOR OF PHILOSOPHY

Major Subject - Plant Chemistry

APPROVED

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In Charge of Major Work.

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Head of Major Department.

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TABLE OF CONTENTS.

INTRODUCTION.

In recent years hydrocyanic acid gas has become a standard fumigant for insects. Its extreme toxicity to most forms of life has made its use general as a destroyer of pests. Many methods have been devised to generate the gas for fumigation purposes. The use of potassium and sodium cyanides treated with sulphuric acid and the use of liquid hydrocyanic acid as sources for the gas were carefully investigated. More recently, various calcium cyanide dusts have been developed which liberate hydrocyanic acid gas when exposed to moist air. "Calcyanide", manufactured by the California Cyanide Company is an example of one of these dusts. It carries fifty per cent hydrocyanic acid.

A fellowship was established by the above Company to study their product. Information for methods of analysis and for methods of studying the toxicity of the gas was desired. A comparison was to be made between the gas obtain- from "Calcyanide" and from liquid hydrocyanic acid. With these problems in view the work was started and the results and conclusions, preceded by a literature study, are presented here.

REVIEW OF THE LITERATURE ON THE TOXICOLOGY OF HYDROCYANIC ACID AND POTASSIUM CYANIDE.

Hydrocyanic acid is often classed as a general protoplasm poison. Its toxic action on animals and on plants has been studied by many investigators since the work of Geppert (1889). This author has described the symptoms of cyanide poisoning in mammals in detail. Very large doses will produce few symptoms in mammals for the animal dies with but slight convulsive movements in a few seconds. With smaller doses respiration is at first increased, vomiting or defecation may occur, the breathing will become more labored but still rapid, convulsions and paralysis will follow, and the respiration will then become more difficult and intermittent. These symptoms, which are described in text books of pharmacology, are said to resemble those of asphyxia. Dontas (1908) recorded similar symptoms in the frog. According to Drzewina (1911), fish exhibit symptoms of asphyxia when placed in cyanide solutions. It seems evident from external observations that a depression of the respiratory nerve center and of all parts of the brain is the main effect of cyanide poisoning in the higher animals.

A thorough study has been made of the action of cyanides on organs and tissues found in the higher animal and a re-

view of this literature gives some idea of the action of hydrocyanic acid on living protoplasm. It was noticed by early workers on this subject that when a red blooded animal was killed with hydrocyanic acid the venous blood retained the bright red color of arterial blood. This condition was explained by the supposition that hydrocyanic acid formed some compound with the hemoglobin. This belief was disproven by the work of Geppert (1889) who demonstrated that the arterial blood was unaffected by the presence of hydrocyanic acid and also that the red venous blood was nothing more than unreduced arterial blood. Zeynek (1901) found that hemoglobin will not unite with cyanides and that oxyhemoglobin unites only after heating several hours at body temperature. Evans (1919) proved that the presence of cyanide did not act on the blood by interfering with the dissociation of oxyhemoglobin. In fact he found that blood from a cat poisoned with potassium cyanide showed a more rapid dissociation of oxygen than did blood from a normal cat. It is now generally recognized that the toxic action of cyanides on vertebrates is not due to the union of the poison with the hemoglobin of the blood.

If the oxygen is not removed from the blood during

cyanide poisoning, it would appear that the living tissues of the body are prevented from performing normal oxidation processes. Geppert's work clearly indicates that one of the measurable actions of hydrocyanic acid on living cells in mammals is a direct decrease in oxygen consumption. This conclusion is supported by Vernon (1906) who found that in isolated kidneys perfused with Ringer's solution the oxygen consumption was reversibly inhibited by cyanides. This author (1910) offers further proof for the inhibition of oxidation in living cells by his work on the action of cyanides on the Cardiac muscle. Evans (1919), working with the isolated tissues of the cat and frog came to the conclusion that there is a reversible chemical reaction between cyanides and the living cells and that there is little evidence that they exert any effects which could not be explained by sudden lack of oxygen. He points out that the nerve centers are more susceptible to cyanides than other parts of the animal and the spinal cord is affected to a less extent than some important nerve centers in the brain. The Linulus heartmuscle is shown by Carlson (1907) to be stimulated by weak concentrations of cyanides and to be depressed by high concentrations. This author does not believe that the primary action of cyanides on the Linulus heart muscle can

be interpreted by the theory that the only action is a prevention of respiration. He also points out that the heart ganglion is more susceptible to cyanide poisoning than is the muscle. Experiments by Richard and Wallace (1908) on the influence of potassium cyanide on protein metabolism would indicate that at least part of the action can be attributed to decreased cell respiration. Hess (1924) and Fleisch (1922) have found that the inhibition of tissue respiration with hydrocyanic acid produces symptoms in pigeons similar to avitamosis.

This brief survey of the effect of cyanides on the higher animals would lead to the conclusion that hydrocyanic acid does reversibly inhibit respiration. The conclusion that hydrocyanic acid affects the respiratory nerve centers and other vital nerve centers also seems justified.

Similar results are obtained when the alkali cyanides or hydrocyanic acid are used to poison lower forms of animals. Shafer (1911) reached the conclusion, from his own work and from a study of the literature, that insects are difficult to suffocate. They could continue to give off small amounts of carbon dioxide when no oxygen was present to be taken up. However, he found that hydrocyanic acid gas increased slightly the value of the respiration quotient for the insects used. He also found (1915) that hydrocyanic acid reversibly reduces

the activities of oxidases, catalases, and reductases in insect tissue.

The view that cyanides reversibly inhibit oxidation has been so generally accepted that cyanides are often used to study the effect of a reduction of the rate of oxidation on living organisms. Budgett (1898) noted that the visible changes in certain protozoa when poisoned with potassium cyanide are exactly reproduced by deprivation of oxygen. Lyon (1902) reached the same conclusion while working with the sea urchin eggs and embryos. Loeb and Westeneys (1913) concluded that potassium cyanide reduced the oxygen consumption of fertilized sea urchin eggs. The process was reversible and it was very similar to a deprivation of oxygen. Hyman (1916) (1919 a) working with the sponge and the Planaria, Child (1919 a) with the Planaria and Allen (1919 a) (1919 b) with the Planaria have found that cyanides reversibly reduce the rate of oxidation.

There are objections to the conclusion that the only action of cyanides on living protoplasm is to reduce the rate of oxidation. Loeb and Westeneys (1913), although they found that potassium cyanide does reduce the rate of oxidation in fertilized sea urchin eggs, point out that the common view that narcosis is due to an interference with oxidation, may

be in error. Since muscular activity in a narcotized animal is low, less oxygen will be consumed and it is a mistake to say that the low oxygen consumption is due to narcosis. They avoid this error by using fertilized sea urchin eggs and they found that chloral hydrate, ethyl urethane, chloroform and various alcohols produce complete narcosis in the fertilized eggs but do not lower the rate of oxidation to any appreciable extent. However, Geppert found that oxygen consumption of mammals was lower when poisoned with hydrocyanic acid even though they do go through a period of high activity and convulsions.

Lund (1918) could not find any decrease in the rate of oxygen consumption in Paramecium. Hyman (1919 a) has found that the Paramecium, except for the amoeba, is the most resistant animal known to potassium cyanide poisoning and it is suggested by this author that the concentrations of potassium cyanide used by Lund were too low. Allen (1919 a) criticizes the use of cyanides in determining the gradient rate of oxidation in living organisms. This author has found that well fed Planaria have a higher rate of oxidation than the starved animals yet the starved animals are more susceptible to cyanide poisoning. However, Hyman (1919 b) (1920) and Child (1919 b) conclude that starvation does not reduce the rate of

oxidation of the parts that are attacked by potassium cyanide. Lund (1921) answers the criticisms of these two authors and concludes, after further work, that "Potassium cyanide even in concentrations which cause cytolysis does not decrease the rate of respiration in Paramecium caudatum" and again that, "The assumption by Child that the rate of respiration in the body wall of the planaria is not primarily affected by feeding, and thatKNC only or primarily affects the body wall and superficial structures is not correct." Further work has been done by Hyman (1922) (1927a,b) and Child (1925) to prove that there is a differential susceptibility.

One of the chief objections which is often raised to the theory that cyanides do directly decrease the rate of oxidation in protoplasm is that many anaerobic organisms and tissues are just as susceptible to cyanide poisoning as aerobic organisms. This objection is explained away by Hyman (1919 a) by pointing out that in many anaerobic organisms the rate of oxidation is just as high as in aerobic organisms but that the former use intra molecular oxidations accomplished by the reduction of sulphates and nitrates, etc. For another example, she states that yeast in the absence of oxygen can carry out intra molecular oxidations by split-

ting sugars into alcohol and carbon dioxide.

The above review of work which has been done on lower animals would again indicate that the action of hydrocyanic acid on living protoplasm reversibly inhibits oxidation processes. Whether or not this is the only action can not be definitely stated.

That cyanides affect lower plants was shown by Schroeder (1907). He found reduction of the rate of oxidation of the fungus, Aspergillus niger, resulted when treated with potassium cyanide. The enzymatic power of bacteria, yeast and fungi have also been found to be inhibited by potassium cyanide. Moore and Willaman (1917) worked on the affect of hydrocyanic acid on the higher plants. They found that a decrease in photosynthesis, a decrease in translocation of carbohydrates, and that a closing of stomata resulted from exposure to the poison. The oxidases and catalases obtained from the plants after fumigation were found to be less active than those obtained before fumigation. They also proved that plant oxidases and catalases are inhibited by hydrocyanic acid gas. It has been shown by these authors that enzymes other than those which are directly connected with the process of respiration such as pepsin, diastase, protease, zymase and rennin are not inhibited in their action by hydrocyanic acid.

However, conflicting observations are reported by other workers. Willstatter, Grassmann, and Ambros (1926) report that some plant proteases can be activated and some inhibited by hydrocyanic acid. Clayton (1919) came to the same conclusion as Moore and Willaman that hydrocyanic acid gas reversibly inhibits respiration in plants.

It is interesting to note that investigators working with plants and animals have often found that anything which will increase oxidation in the tissues or increase the activity of life processes will make living protoplasm more susceptible to hydrocyanic acid poisoning. For example, an increase in temperature on insects or an increase in temperature or intensity of light on plants will decrease their resistance to the poison. This was found to be true of the weevil and the cockroach which were used in the toxicity experiments recorded in this paper. Temperatures which were not low enough to cause permanent injury, and yet low enough to make the insect sluggish, made it more resistant to hydrocyanic acid poisoning. This point was determined only in a qualitative way but the same relationship has been noted between temperature and susceptibility by most workers. To quote from Komp (1921) "It was found, as would be expected, that the effectiveness of the fumigation was much increased

by an increase in temperature," and again, "The failure to kill the aphids at a low temperature will explain the rather high percentage of hydrogen cyanide necessary to produce killing effects in greenhouses, where as a matter of safety to the plants during fumigation the temperature is kept low." The relationship between temperature and susceptibility would again lead one to think that the reaction is a chemical one which probably includes respiration processes.

From his work with liquid hydrocyanic acid Bodine (1924) believes that, "The physiological action of HCN and its salts appear to be due (a) to the ease with which HCN molecules penetrate living cells and then ionizing, exert their influence by means of H ions and CN ions; (b) to the weakness of HCN as an acid, which permits at neutrality or even slight alkalinity the presence of a considerable amount of free HCN molecules in the presence of their salts; (c) to the specific effects occasioned by its chemical activity."

Since the work of Mathews and Walker (1909) by which they showed that very small amounts of potassium cyanide checked or prevented the spontaneous exidation of cystein in neutral or alkaline solution, much work has been done on the inhibition of all types of oxidation processes by hydrocyanic acid or the alkali cyanides. Generally a retardation of oxi-

dation processes by hydrocyanic acid is noticed although in the presence of some metals acceleration of the process results. Warburg (1923) came to the conclusion that the anticatylitic action of hydrocyanic acid to oxidation with HIO_3 is due to the presence of iron and that hydrocyanic acid is a specific poison for intracellular iron. He is supported by Toda (1926) who worked with the action of hydrocyanic acid on the oxidation of oxalic acid by HIO_3. Wieland and Fischer (1926), however, do not subscribe to the above theory since they worked with purified reagents and the rate of oxidation was still reversibly inhibited by hydrocyanic acid.

We can conclude that hydrocyanic acid may reversibly inhibit oxidation processes whether in living tissues or in laboratory reactions. That it may accelerate in small concentrations and retard in higher concentrations is also probably true and there are indications that hydrocyanic acid may affect life processes other than respiration.

REVIEW AND DISCUSSION OF THE LITERATURE ON METHODS OF ILLUSTRATING AND INTERPRETING THE ACTION OF POISONS.

Paul and Krŏhig (1896) noticed that when microorganisms were treated with a disinfectant death did not occur at the same instant for all the organisms. Madsen and Nyman (1907) secured data on the action of mercuric chloride and heat on anthrax spores. If the number of surviving bacteria in one of their experiments was plotted against time a curve which showed agreement with the curve for a mono-molecular reaction resulted. Chick (1908) (1910) did further work on the action of disinfectants on microorganisms. She concludes (1908), "An ideal case of disinfection such as that of anthrax spores, may therefore be supposed, with experimental support, to proceed in accordance with the equation $\frac{dn}{dt} = kn$, an equation exactly similar in form to that expressing the course of a unimolecular reaction of the first order." Many workers began to view disinfection as a chemical reaction and that the microorganism and the disinfectant could be considered as the reagents. It is important to note that all experimental results did not conform to this theory. A study of what this type of curve would mean for a

biological reaction such as disinfection ought to indicate the validity of Miss Chick's assumption.

There are two ways which are generally used to illustrate rate of killing by toxic agents. The curve used by Miss Chick is an example of what may be called a time course curve in which the number of surviving bacteria are plotted against time. This is the type of curve generally used by the bacteriologist. The physiologist also uses the time course curve but he usually plots the percent kill against time. The most common curve obtained by the physiologist is the sigmoid or "S" shaped curve. For example, Brooks (1918) finds the sigmoid form to be the normal shape for hemolysis. Schakell (1925) states, "The relation between dosage and effect is represented graphically by an S curve. It has been pointed out that the latter is a cumulative frequency curve of individual sensitivities of the organism in a given population. It is proposed, therefore, to speak of this curve as the dose-effect (or toxicity) ogive."

The second type of curve in common use is the rate curve whose ordinates are proportional to the number of individuals killed in a unit time. This means of expressing results is used by Loeb and Northrop (1917) in their work

on the effect of temperature on fruit flies. This mortality rate curve is very commonly used in the study of vital statistics.

As Brooks (1918) points out, "If the rate of hemolysis were uniform the time curve would be a sloping straight line (the integral curve, a, Fig. 2), while since its tangent or slope is the same at every point, the differential or mortality curve would be a straight line parallel to the axis of the abscissae." To show the relationship between the time course curve and the mortality rate curve, one of Loeb and Northrop's (1917) experiments on fruit flies (Table 12, page 119) is plotted in two ways. Fig. 1 is the time curve for this experiment and Fig. 2 is the corresponding mortality rate curve. The latter curve is not an exact differential of the former since it was assumed that the tangent to any point on the curve would pass through the next plotted point. However, the common method of determining the mortality rate curve is to plot the percent of organisms dying during a set interval against time.

The conclusion that Miss Chick's results indicate a monomolecular reaction is much debated. Loeb and Northrop (1917) criticising Miss Chick's assumption, state, "She was probably led to such an assumption by the fact that the ascending branch of the mortality curve in her experiments was

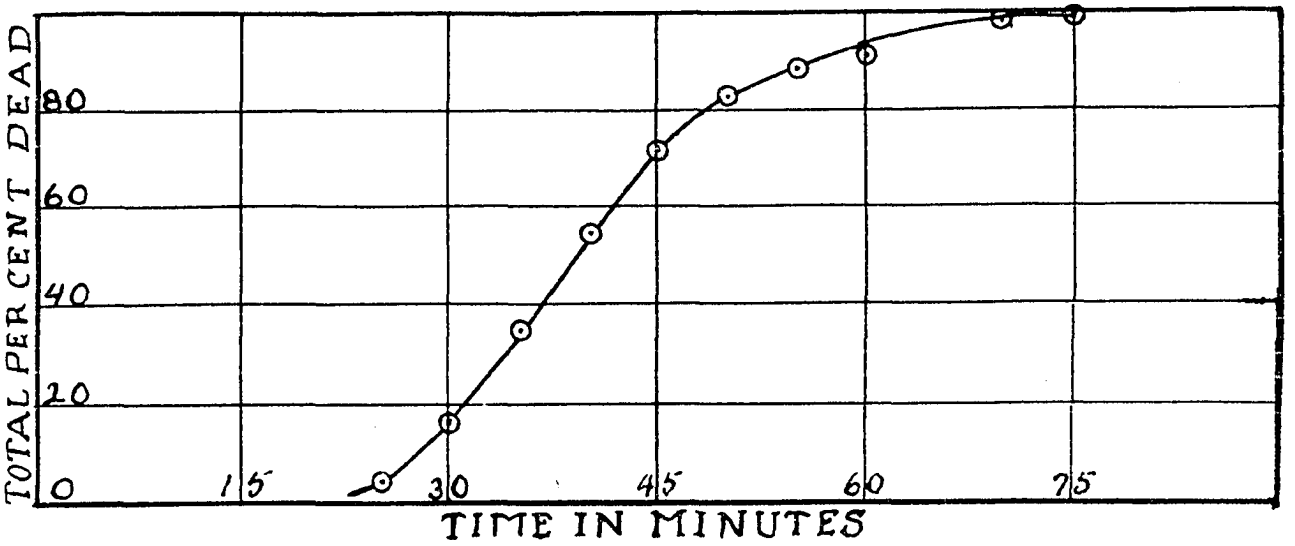

Fig. 1. Temp. 39.45°C. on fruit flies.

(Loeb and Northrop)

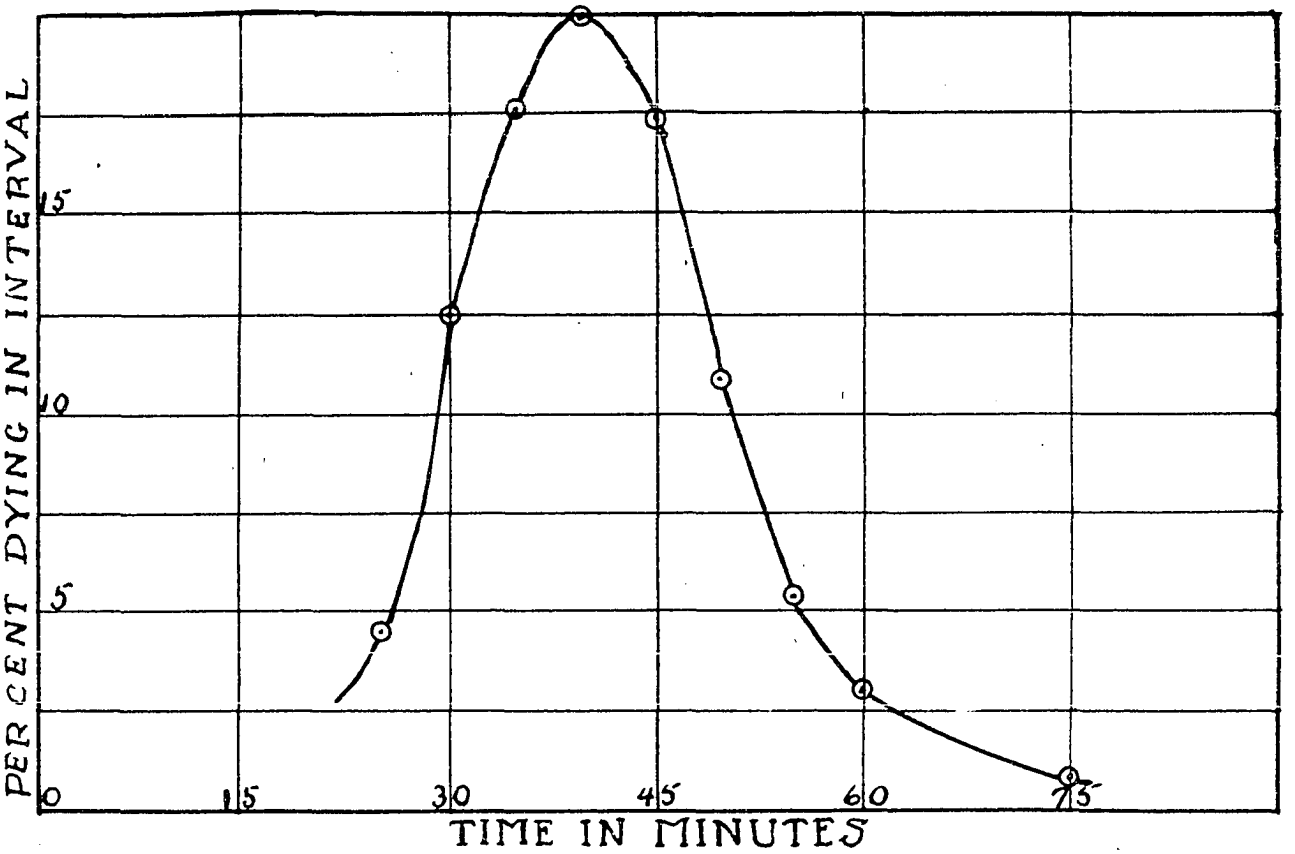

Fig. 2. Temp. 39.45 C. on fruit flies.

(Loeb and Northrop)

generally very steep. The agencies used by her for killing the bacteria were so powerful that the ascending branch became almost a verticle line, thus escaping detection. Hence she noticed usually only the less steep descending branch which could be interpreted as a monomolecular curve for the reason that her experiments lasted only a short time." Brooks (1918) concludes, "The course of such a process as hemolysis is very largely dependant upon variations in resistance among the different individuals, and secondarily upon the course of the fundamental reaction." and also, "Unnatural assumptions would be requisite for the explanation of a resemblance between the course of such processes in general and that of a monomolecular reaction". Smith (1921) working with Botrytis spores exposed to the action of 0.4 percent phenol obtained a time curve for the surviving numbers which was sigmoid in shape. He concluded that with the same suspension it was possible to obtain either a logarithmic or a sigmoid shaped curve depending in the concentration of phenol used. He also found that by using progressively younger or less resistant spores exposed to the same concentration of phenol he could obtain identical results. Fulmer and Buchanan (1920) while working with yeast came to the conclusion that, "Variations in resistance of individual cells and the distribu-

tion of such variations must be regarded as of fundamental importance in accounting for rates of death of microorganisms."

Another means of illustrating toxic action is to plot the concentration of poison used against the time interval necessary to produce one hundred percent kill. Campbell (1926 a) plots "speed of toxic action", against concentration. By speed of toxic action he means one hundred divided by the time necessary to produce one hundred percent kill. He points out that this curve "has nothing to do with variation in individual susceptibility, the influence of which, is or should be, eliminated by poisoning a sufficient number of individuals at each dosage."

There are two general methods used by the Economic Entomologist to compare the effect of a toxic gas on different insects and also to compare the relative value of various toxic gases on one insect. One of these methods is to find the minimum lethal dose. For example, Neifert and Garrison (1920) when making a comparison between phosgene and hydrocyanic acid found "the minimum lethal dose of phosgene with the minimum time to kill as compared with that of hydrocyanic acid." Quoting again from their paper, "The minimum lethal dose of arsine for certain periods of time as compared with that of hydrocyanic acid,

is given in Table 3." The other method is to hold the time of exposure constant and to determine the concentration of the toxic gas necessary to produce one hundred percent kill.

Tattersfield and Coworkers (1923) (1925 a,b) (1927) working with the affect of different sprays on Aphis Rumicus L have developed another method for comparing the toxicity of chemicals. If it is true that each insect receives a similar dosage, they state (1923) "For the immediate purposes of our investigation the factors varied were the poison and its concentration, so that provided that the foregoing assumption is correct, the proportion of dead to numbers sprayed will at each concentration give a measure of the toxicity at that concentration. If a number of chemical substances are tested at different strengths, curves can be plotted indicating how toxicity varies with the concentration and chemical constitution. From some suitable point upon each of these curves a direct comparison of toxicity between the chemical compounds can be made. Statistically the 50 percent death point, that is the concentration of poison which kills 50 percent of the insects sprayed, is the best. If, for example, the concentration of a standard poison such as nicotine giving a 50 percent mortality is known, the ratio of this amount to the amount of another

substance giving the same mortality may be regarded as an insecticidal index for that substance, while if the curves are continued from their lower to their upper limits, indications will be obtained of the strengths that the insects can sustain without injury, and those required to kill 100 percent." This method is practically the same as the one used to study the toxic effect of gases by holding the time constant and then varying the concentration, except that, in the latter case, the point of comparison is the one hundred percent mark. The curves which are plotted by Tattersfield would also indicate the effect of varying the concentration.

MATERIALS AND METHODS.

A. Source of Materials.

The hydrocyanic acid gas for this work was obtained from "Calcyanide", furnished by the California Cyanide Company and from liquid hydrocyanic acid prepared by the action of sulphuric acid on sodium cyanide. (Ziegler 1921). It is interesting to note that the liquid acid after it had been redistilled over calcium chloride several times kept without decomposition for nine months. During most of this time the liquid was kept dry in a closed container which was cooled by ice. During the month of September it was allowed to warm up to room temperature. During the entire time it was protected from sunlight and at the end of nine months a slight yellow coloration was noticeable. Acknowledgement is made to L. C. Heckert who prepared and donated the liquid hydrocyanic acid for this work.

The cockroaches were obtained by trapping them in bottles, all being caught in the one locality. Insects were used which were apparently in good condition and of approximately the same size. The weevils were raised in bottles containing fresh clean wheat and again results are based only on insects which were in good condition. The rats

were donated by the Physiological Chemistry Department and were those fed on a standard ration in preparation for experimental work.

B. Description of the Apparatus.

Fig. 3 is a diagram of the apparatus used to determine the toxicity of hydrocyanic acid gas generated from "Calcyanide." To illustrate the operation of the apparatus the course of the air and gas will be traced. As indicated by the arrow, air enters the generating train at (a). Tube (c) acts as a pressure regulator. It consists of a Carius tube with mercury in the bottom (the darkened portion) and a head of water through which excess air bubbles. Screw clamp (b) aids in regulating the flow of air. In bottles (d) and (e) the air bubbles through sulphuric acid of sufficient density to produce sixty percent humidity. It is then forced through glass wool in bottle (f) to take up acid spray. Next it flows over sixty grams of "Calcyanide" distributed through tube (g). Here the moist air generates hydrocyanic acid from the easily hydrolyzed calcium cyanide. As the gas air mixture is forced through the flowmeter (h) the rate of flow can be determined and fixed at some mark. For the experiments recorded in this paper, a rate of forty

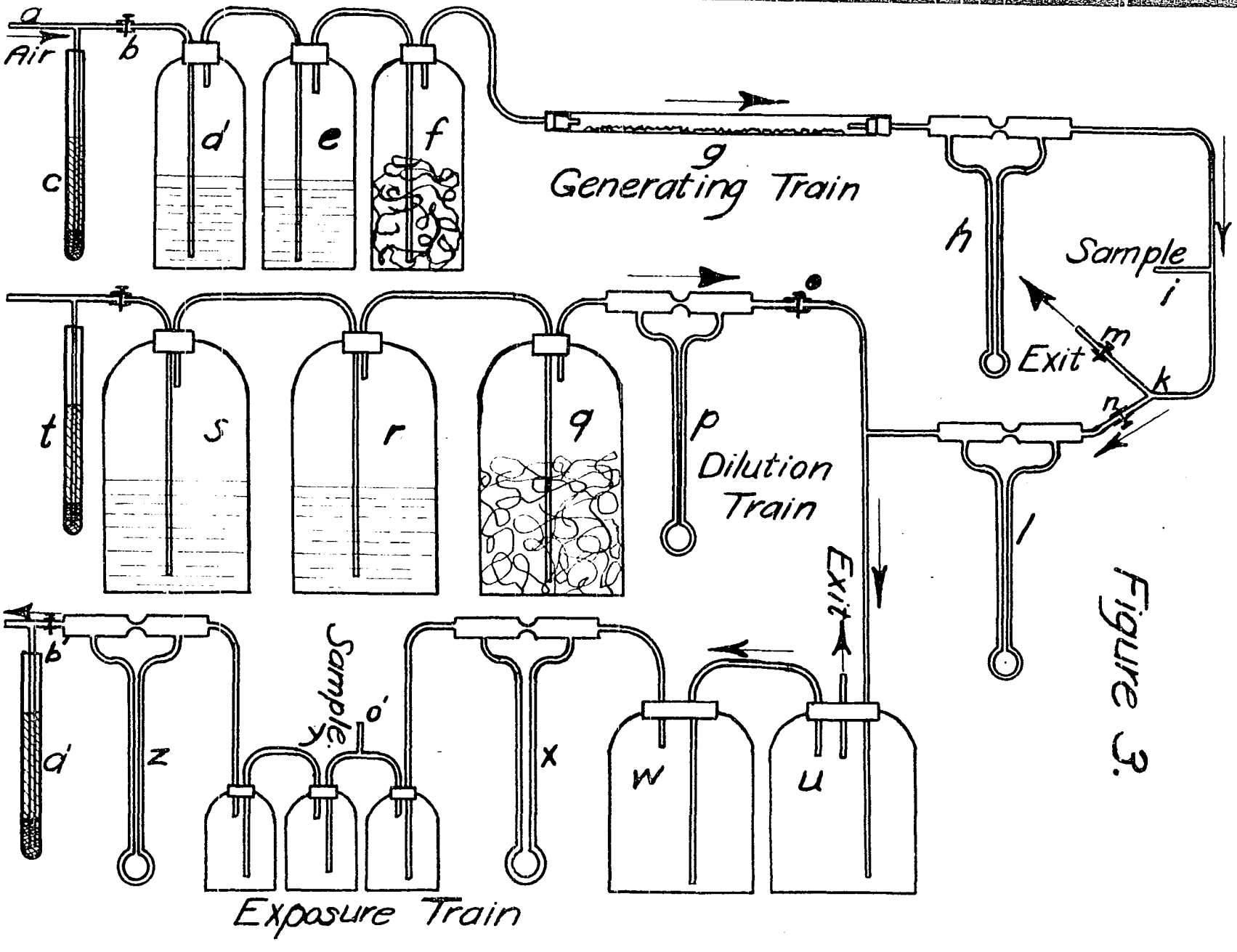
a
Air
b
c
d
e
f
g
Generating Train
h
Sample
i
Exit
m
k
n
t
s
r
q
p
Dilution
Train
l
Exit
b'
d'
z
Sample
o'
x
w
u
Exposure Train

Figure 3.

liters per hour was generally used. The concentration of hydrocyanic acid can be determined by taking a sample through tube (i).

It is often necessary to dilute with more air, hence the (y) tube (k) permits part of the gas to be forced out of the laboratory into the open and part through flowmeter (l). The rate of flow can be regulated by screw clamps (m) and (n). If it is necessary to dilute the gas with more air in order to obtain a desired concentration, screw clamp (o) may be opened which will connect the dilution train with the system. The direction the air flows through the dilution train is indicated by arrows. Again (t) is a pressure regulator, (s) and (r) contain sulphuric acid of a density to give the desired humidity, (q) contains glass wool to take up the acid spray, and (p) measures the rate of flow of the diluting air.

The diluted air hydrocyanic acid gas mixture now passes into mixing bottle (u). Excess gas mixture is permitted to escape into the open through (v). The amount which is needed for the experiment is drawn by a water suction pump into mixing bottle (w), through flowmeter (x), into exposure bottles (y) and through flowmeter (z). Tube (a^1) is a suction regulator. The rate of flow can be controlled by screw

clamp (b^1) and can be determined by flowmeters (x) and (z). Any leak in the exposure train can also be detected since the two flowmeters should indicate the same rate of flow. Samples for analysis may be taken from o^1.

In all the experiments on toxicity when "Calcyanide" was used, 60 grams was distributed evenly in tube (g). This tube had a three-fourth inch bore and a length of four feet. Air (60% humidity) was passed continually through the tube, usually at the rate of forty liters per hour for three to four days. The first and second day the gas mixture obtained was generally diluted with air until the required concentration of hydrogen cyanide was reached. In most cases no dilutions were made the third and fourth day.

In order to obtain hydrocyanic acid gas from the liquid acid another generating train was substituted in the apparatus described above. Air dried by pressing it through calcium chloride and concentrated sulphuric acid was bubbled through liquid hydrocyanic acid kept at a definite temperature. In most of the experiments the liquid was kept at approximately zero degrees centigrade by surrounding the container, which was inserted in a thermos bottle, by ice. The air saturated with hydrocyanic acid gas then passed through

a flowmeter which can be considered as replacing (1) in the diagram. The rate of flow could then be determined and the gas mixture diluted to the desired strength as before. Since the vapor pressure of liquid hydrocyanic acid is known for various temperatures (Perry and Porter (1926)) it is possible to calculate approximately the amount of gas picked up by the air and dilutions made accordingly.

C. Methods of Analysis.

Two methods of analysis were used in this work, the thiocyanate method of Francis and Connell (1913) and the ordianry Licbig method. Since the former method was modified somewhat it will be described in detail. The application of the Liebig method to this work will also be discussed.

1. Colorimetric Method for the Determination of Hydrocyanic Acid.

The gas mixture is asperated through fifty cubic centimeters of six normal ammonium hydroxide. When the concentration of hydrocyanic acid is as high as ten to fifteen thousand parts of hydrogen cyanide per million parts of air gas mixture, a one to two liter sample is best. The amount of gas mixture taken for a sample is increased as the concentration of hydrogen cyanide drops. For two hundred parts per million a ten liter sample is recommended.

Five cubic centimeters of freshly prepared yellow ammonium sulphide is added to the sample and the mixture is evaporated to dryness on a water bath. The residue is taken up with ten to fifteen cubic centimeters of hot water and is barely acidified with sulphuric acid. One drop of six normal sulphuric acid is sufficient. The sample is filtered through quantitative filter paper to remove the free sulphur. One half of one cubic centimeter of six normal sulphuric acid is added and heat is applied for five minutes by placing the container in boiling water. The sample is filtered to remove the free sulphur which comes down, cooled, and refiltered if necessary to remove all traces of free sulphur. It is made up to volume in a volumetric flask. When the analysis is above five thousand parts per million it is best to make up from one hundred to two hundred cubic centimeters; below five thousand, accurate dilutions can be made from fifty cubic centimeters. The subsequent dilutions are made in fifty cubic centimeter Nessler tubes. Two drops of a five percent ferric chloride solution is added and the color compared to standards.

The standards are made from a standard potassium cyanide solution, one cubic centimeter of which is equivalent to two milligrams of hydrocyanic acid. Two cubic centimeters of the standard is pipetted into an evaporating

dish and diluted with about ten cubic centimeters of distilled water. Five cubic centimeters of yellow ammonium sulphide is added and the sample is treated exactly as the unknown. The solution is made up in a two hundred cubic centimeter volumetric flask. Enough standard is made to measure fifteen, twenty, twenty-five, thirty, thirty-five, forty, forty-five and fifty cubic centimeters respectively into fifty cubic centimeter Nessler tubes. Two drops of a five percent ferric chloride solution is added to each tube. The color should be a delicate red and should be easily determined.

The concentration is expressed in parts of hydrogen cyanide in a million parts of gas air mixture. The gas is assumed to be perfect and no correction is made for temperature or pressure.

Notes on the Colorimetric Method of Analysis for Hydrocyanic Acid Gas.

(1) After the sample has been taken in the ammonium hydroxide it should not be left standing for any length of time. Standing over night will often reduce the percentage of hydrocyanic acid to one-half its correct value.

(2) It was found that freshly prepared yellow ammonium sul-

phide gave the best results.

(3) The acid concentration affects the color very much. Francis and Connell (1913) recommend that one, "Add one-half cc. dilute hydrochloric acid and boil 5 minutes." They also state that "If too much acid is present, the solution will be lemon yellow. If alkaline, the iron will be precipitated, but this condition may be corrected by the addition of a few drops of acid." The lemon yellow color was observed when too much acid had been added or when the solutions were made up too dilute for comparison with the standards. Best comparative results were obtained when the directions given in the modified method were followed. To avoid the presence of an acid and its salt, Johnson (1916) extracted the thiocyanate residue in the form of the potassium salt with acetone. He removed the acetone by evaporation and used the potassium thiocyanate directly instead of converting it to the free acid.

(4) Prolonged heating in the boiling water to bring down the sulphur is not desirable. According to Viehoever and Johns (1915), "In boiling an acid solution of a thiocyanate, some free thiocyanate is lost because it is volatile, the boiling point of the acid being 85°".

This factor is not as important in the modified method since the solution is not boiled for five minutes as was done by Francis and Connell. It was also found by Johnson (1916) while studying this method that, "When a solution containing 3 cc. of the standard thiocyanate was boiled vigorously under the condition of the method for ten minutes less than 0.2 cc. was lost."

(5) In order to make a comparison of the colors all of the free sulphur must be removed. This can be done by reheating in the water bath if necessary. Very little change in the final percentage of hydrocyanic acid was noted after a sample had been heated for two five minute periods. Menaul and Dowell (1920) heated the residue, after the solution had been evaporated to dryness on the water bath, to 130°C. The sulphur was then rendered insoluble. This practice is questionable since it was found that if the sample was allowed to remain on the water bath for any length of time after it had reached dryness the resulting analysis was always very much too low.

2. Discussion of the Liebig Method for the Determination of Hydrocyanic Acid.

The Liebig method is much simpler and more

dependable than the colorimetric method outlined above. By using a microburette and a solution of silver nitrate, one cubic centimeter of which equals one to two milligrams of hydrogen cyanide, concentrations as low as two hundred parts per million can be determined with accuracy.

A ten liter sample of the gas air mixture is drawn through bubbling bottles filled with fifty cubic centimeters of a one percent potassium hydroxide solution. The strength of the potassium hydroxide is varied according to the rapidity with which the gas is drawn through it and also to the concentration of hydrocyanic acid gas in the gas air mixture. The one percent solution was found to be best for most of the work.

The sample may be titrated directly in the bubbling bottles or transferred to another vessel. The addition of five cubic centimeters of a two percent potassium iodide solution tends to make the end point sharper but is not absolutely necessary. It has been the practice of some workers when determining concentrations as low as two hundred parts of hydrocyanic acid gas per million to reduce the normality of the silver nitrate. For example, Komp (1921) used 0.001 N silver nitrate for a titrating solution. It would be difficult to determine the

end point when using such a dilute solution since it depends upon the appearance of turbidity due to precipitation. The use of a stronger solution and a microburette makes it possible to get a good end point and at the same time retain the accuracy sought by using a more dilute titrating solution of silver nitrate.

A comparison of the colorimetric method and the Liebig method for determining the concentration of hydrocyanic acid gas is given in Fig. 4. The gas was generated from "Calcyanide" and the concentration of the diluted gas mixture was determined at intervals by both methods for a period of seven hours. The circles indicate results obtained by the Liebig method and the crosses give the concentrations obtained by the colorimetric determination.

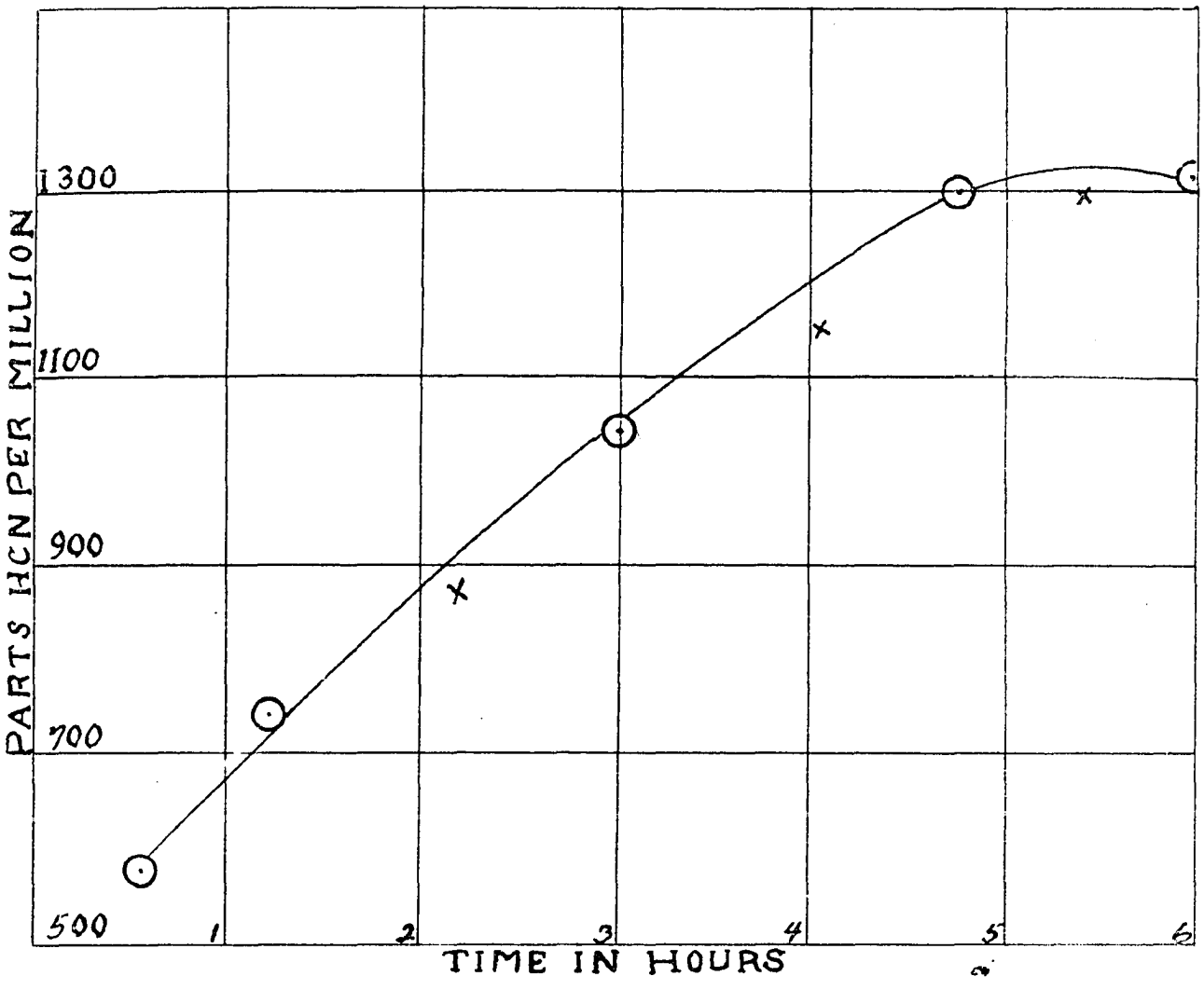

Fig. 4.

⊙ - Results obtained by the Liebig method of analysis.

X - Results obtained by the colorimetric method of analysis.

The hydrocyanic acid gas was generated from "Calcyanide".

D. Experimental Procedure.

The Cockroach

(Blatta orientalis)

Seven exposure bottles were kept in the train. Ten or more cockroaches were quickly put into a bottle as soon as the concentration of hydrocyanic acid gas had been determined. The bottles were small (250 cc.) and the gas was drawn through them at such a rate (20-30 liters per hour) that any error introduced by leakage during this process was negligible. The groups of cockroaches were exposed different lengths of time. When they were removed from the exposure train they were immediately put into clean Erlenmeyer flasks. Their condition was noted at intervals of twenty-four and forty-eight hours after the exposure to the gas. They were placed in one of three categories:

Recovered. - Cockroaches which could walk.

Moving. - Cockroaches which could move any of their appendages but not likely to recover.

Not Moving. - Cockroaches apparently dead.

The time after exposure which is taken for the final determination of the condition of the cockroaches is im-

portant. Immediately after exposure they will all appear dead. It was found after some experience that, although there is often little difference between the condition of the insects after twenty-four hours and after forty-eight hours, the forty-eight hour period gave the best comparative results. It was also found that those insects which were classed as moving, after they had been given forty-eight to recover, died in a short time. For this reason, when recording mortality, the number moving and the number not moving were counted as dead.

It is true that if a larger number of cockroaches had been used the time interval necessary to produce one hundred percent kill would have been lengthened. However, approximately 800 cockroaches were used to determine the time intervals for different lethal concentrations and the results obtained are probably not far from correct. The time course curves for the cockroach are more of an approximation since they were only obtained incidentally while seeking the time for one hundred percent kill. Usually the whole range from zero to one hundred percent kill for one concentration was not investigated.

An example of the way the results were recorded is given in Table I.

TABLE I.

Parts HCN per million	Minute Exposed	Number of cock-roaches	Number recover-ed	Number Moving	Number not Moving	Hours after exposure
869	15	10	9	0	1	48
869	30	10	5	0	5	48
869	45	10	2	2	6	48
869	60	10	1	1	8	48
869	75	10	0	0	10	48

The Weevil

(C. oryzae).

The same procedure was followed with the weevil as was outlined for the cockroach except that one hundred weevils were put in each exposure bottle. It is desirable to keep a control bottle or two if the condition of the weevil is not known. A class of insects which are near the end of their life cycle or which have been kept under unfavorable conditions will often die naturally during the period they are under observation. They will also be more susceptible to the gas and cannot be compared to a group of more re-

sistant ones.

An example of the way the results were recorded is given in Table II.

TABLE II.

Parts HCN per million	Minutes Exposed	Percent Kill
10,460	150	5
10,460	210	30
10,460	270	66
10,460	330	95
10,460	360	100
10,460	420	100

The Rat

In the rat experiments two exposure bottles were used of sufficient size to hold a medium sized rat. One or two rats were exposed at one time. Upon removal from the exposure bottle respiration was taken by holding a mirror to the nose and the heart beat was also recorded. These factors were noted at intervals until it was certain that the rat had recovered or was dead. The results were kept as

in the following table.

TABLE III.

Parts HCN per million	Weight of rat in grams	Minutes Exposed	Kill
270	70	10	-
270	70	15	-
270	89	15	-
270	70	20	+
270	90	20	-
270	70	20	+
270	60	20	+
270	91	20	+
270	96	20	-
270	93	23	-
270	55	25	+
270	83	25	+
270	70	30	+

All of the toxicity experiments recorded were carried out at a room temperature with a maximum variation of from 25° to 30°C.

DISCUSSION OF RESULTS.

Methods of Studying the Toxicity of Hydrocyanic Acid.

Since there are so many methods used to express toxic action the attempt will be made to present the experimental data in such a way that the relationship between the different methods will be apparent. In the review and discussion of the literature on toxicity curves it was pointed out that the Economic Entomologist does not use the time course curve, the mortality rate curve, or a lethal concentration time curve in his study of fumigants. It will be shown that such methods would be of practical value to him for determining the type of fumigant to use and the most efficient dosage.

A time course curve for an experiment, where weevils were exposed to a concentration of ten thousand four hundred and sixty parts of hydrogen cyanide gas per million parts of air gas mixture, is plotted in Figure 5. The data for this experiment is summarized in Table 2, page 41. The curve is sigmoid, a shape which is common in biological work.

An equation which fits this curve approximately is

$$y = \frac{0.1\, e^{5/3x}}{0.9 + 0.001\, e^{5/3\, x}} \qquad (1).$$

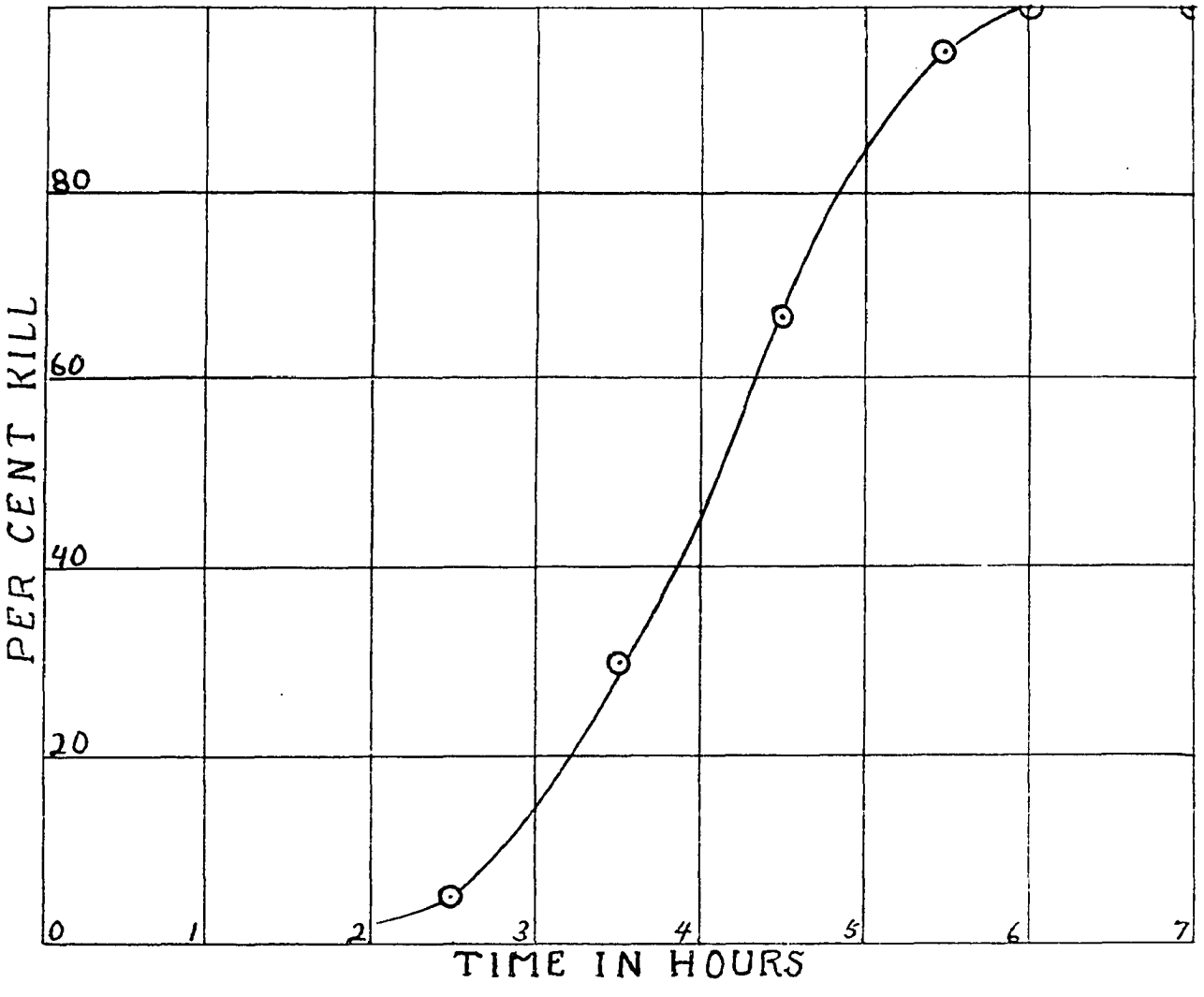

Fig. 5. This is a time course curve for the weevil exposed to 10,460 parts HCN to a million parts of gas-air mixture. One hundred weevils were used to determine each point.

This equation is only given to show the mathematical relationship between the above curve and the mortality rate curve. Differentiation of (1) yields equation (2).

$$\frac{dy}{dx} = \frac{5(0.1\ e^{5/3x})}{3(0.9 + 0.001\ e^{5/3x})^2} \qquad (2).$$

Now if $\frac{dy}{dx}$ is plotted against x the curve in Figure 6 is obtained which is a mortality rate curve for an experiment which is described by equation (1). It indicates the rate at which the organisms are dying at any particular time.

The point which was raised by Smith (1921) that the shape of the time course curve is affected by the concentration of poison used, is further substantiated by the action of different concentrations of hydrocynic acid gas on the cockroach. Curves a, b, and c. in Figure 7 illustrate the effect of progressively lowering the concentration on the cockroach. To obtain curve a, 3523 parts of hydrocyanic acid gas per million was used, to obtain b, 689 parts and to bbtain c, 407 parts. It was pointed out in the description of the experimental procedure that these curves are at best only approximations since but ten to fifteen insects were used for each point. The three experiments plotted gave the smoothest curves and are used only to illustrate the

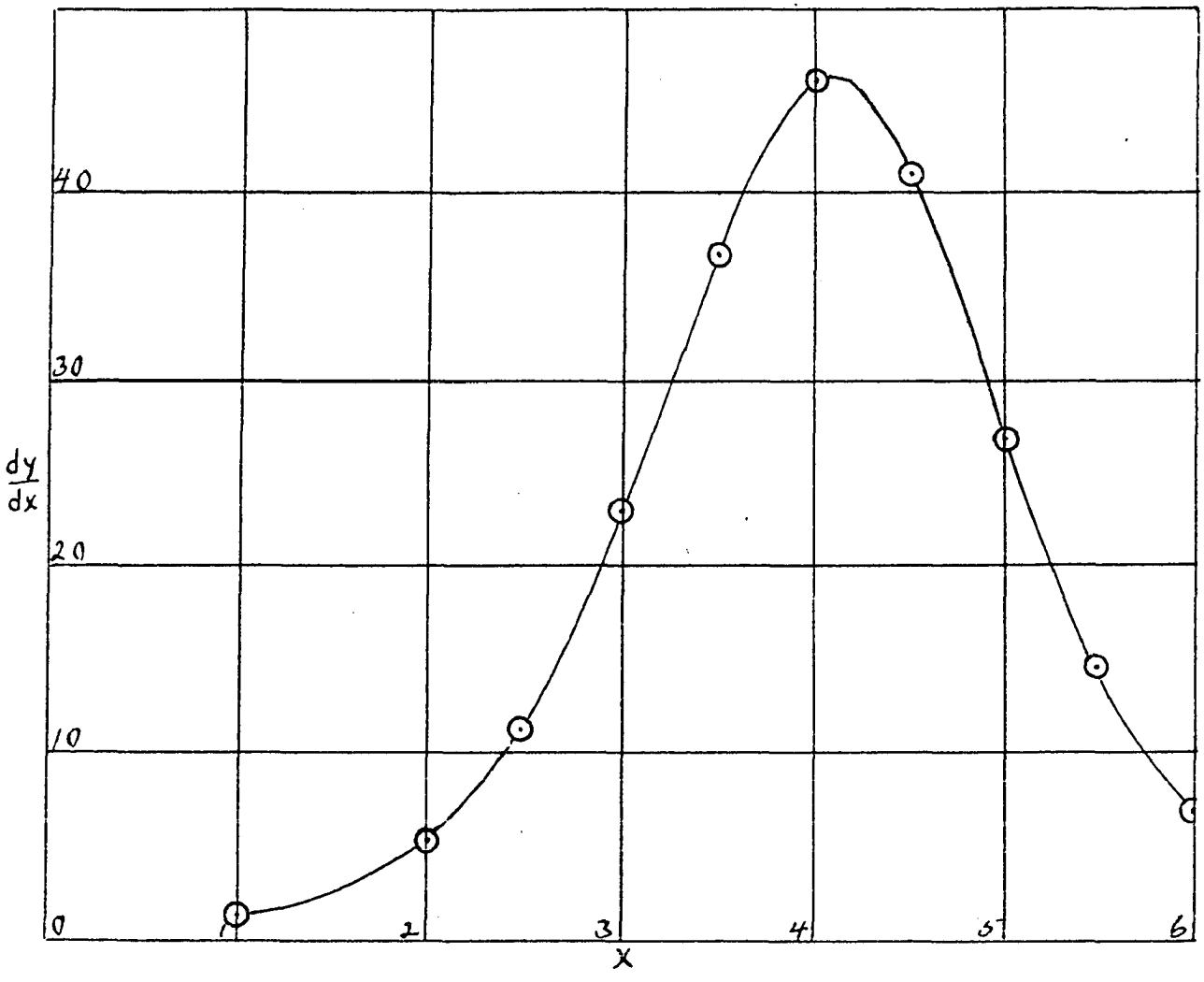

Fig. 6. This is a mortality rate curve obtained from the equation $\frac{dy}{dx} = \frac{5}{3} \cdot \frac{0.1\, e^{5/3x}}{(0.9 + 0.001\, e^{5/3x})^2}$.

$\frac{dy}{dx}$ is plotted on the ordinate and x on the abscissae.

effect of a change of concentration. In every experiment the slope of the curve increased as the concentration increased. This change in slope would be expected since the time between zero and one hundred percent kill becomes greater as the concentration of the gas decreases.

Undoubtedly the weevil curve in Figure 5 is more accurate than the cockroach curves since one hundred insects were used to obtain each point. The effect of shortening the time ordinates used to plot this curve is shown in Figures 8 and 9. If the sigmoid shaped curve was always obtained then an increase in concentration ought to change its course in the same way as a shortening of the time ordinates.

There are many other factors which may cause variations in the shape of a time course curve. In bacteriological work there is a chance of a change in the reaction of the medium. In feeding experiments, factors such as stomach contents, rate of excretion, absorption, etc., undoubtedly would affect the course of the curve. The factor which is usually stressed is the variation and distribution of resistances. The shape of the curve is probably due to a summation of all the factors which enter into the reaction of an organism with its poisoned environment.

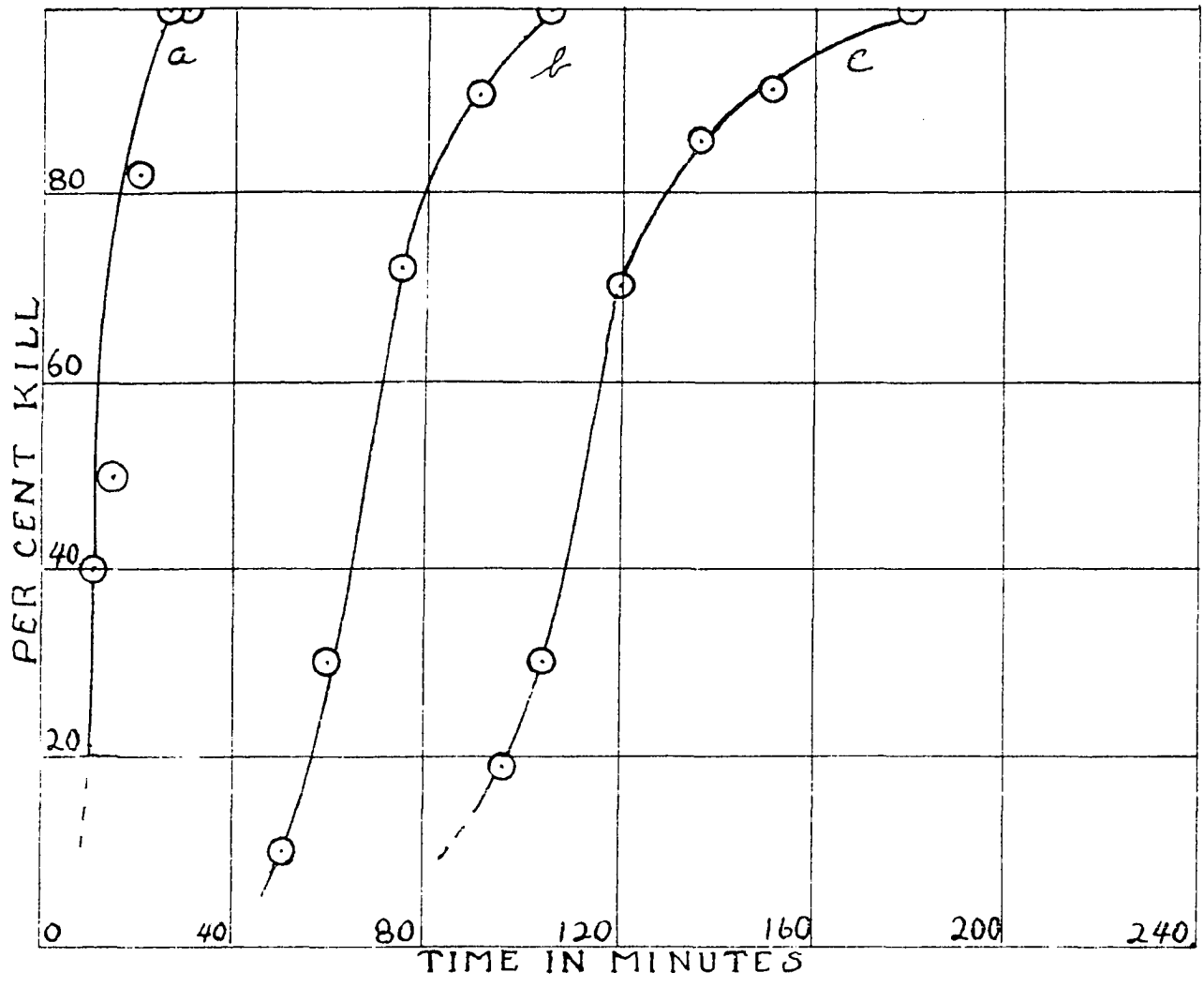

Fig. 7. The effect of concentration on the time course curves for the cockroach is shown. To obtain curve (a), cockroaches were exposed to 3523 parts of HCN per million parts of air-gas mixture, to obtain (b), 689 parts, and (c), 407 parts.

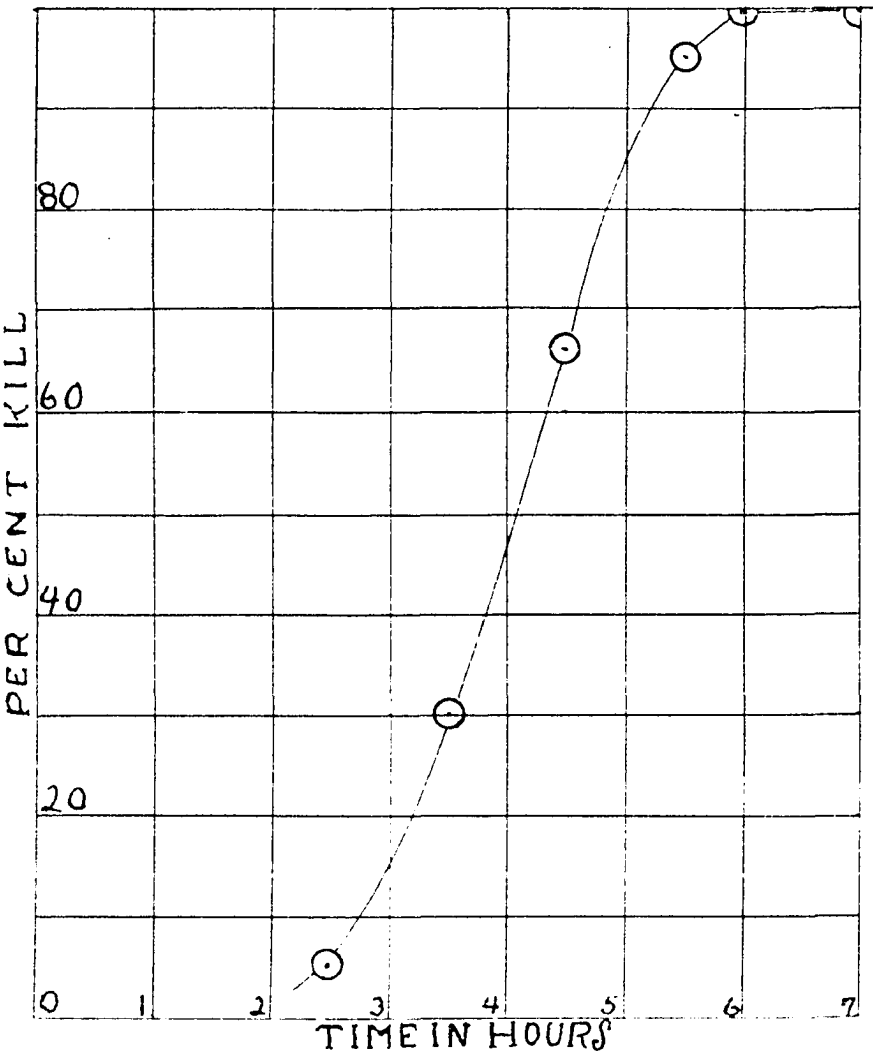

Fig. 8.

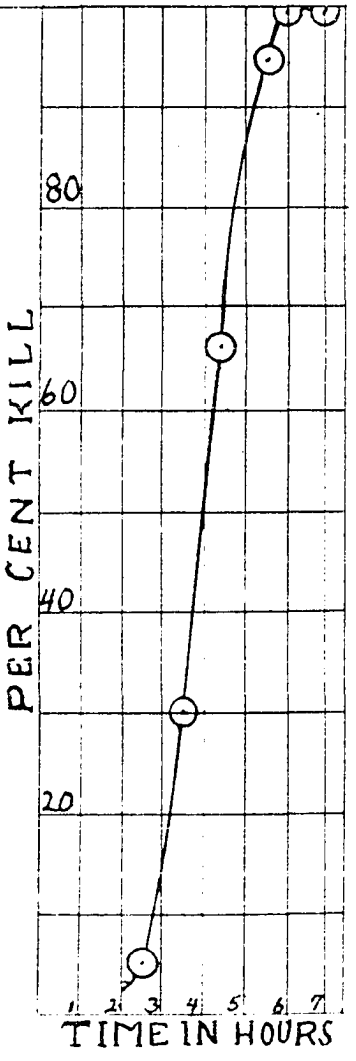

Fig. 9.

Figures 8 and 9. The effect of shortening the time ordinates used to plot Figure 5 is shown.

The curve in Figure 10 is an example of a variation from the sigmoid curve shown in Figure 5. The former curve was obtained in the same way as the latter except that the weevils were exposed to only 381 parts of hydrocyanic acid gas per million. At such a low concentration the time used is so long that the cause of death may be different than at higher concentrations. Variations in resistance would probably be more apparent.

Such curves should be of value to the Economic Entomologist in his study of the susceptibility of insects to a fumigant by giving him a summation of all these factors. The rate at which the insects die would be of interest to him for it would be an aid in picking the most desirable concentration to use. There appears to be a scale developing in California which is resistant to hydrocyanic acid under present fumigation conditions. Time course curves and mortality rate curves would be an aid in determining the presence of an immune species.

Another method for determining the action of poisons was used while making a comparison of the action of hydrocyanic acid gas as a poison for a red blooded animal such as the rat, and different insects such as the cockroach and the weevil. This was done by finding the time interval necessary

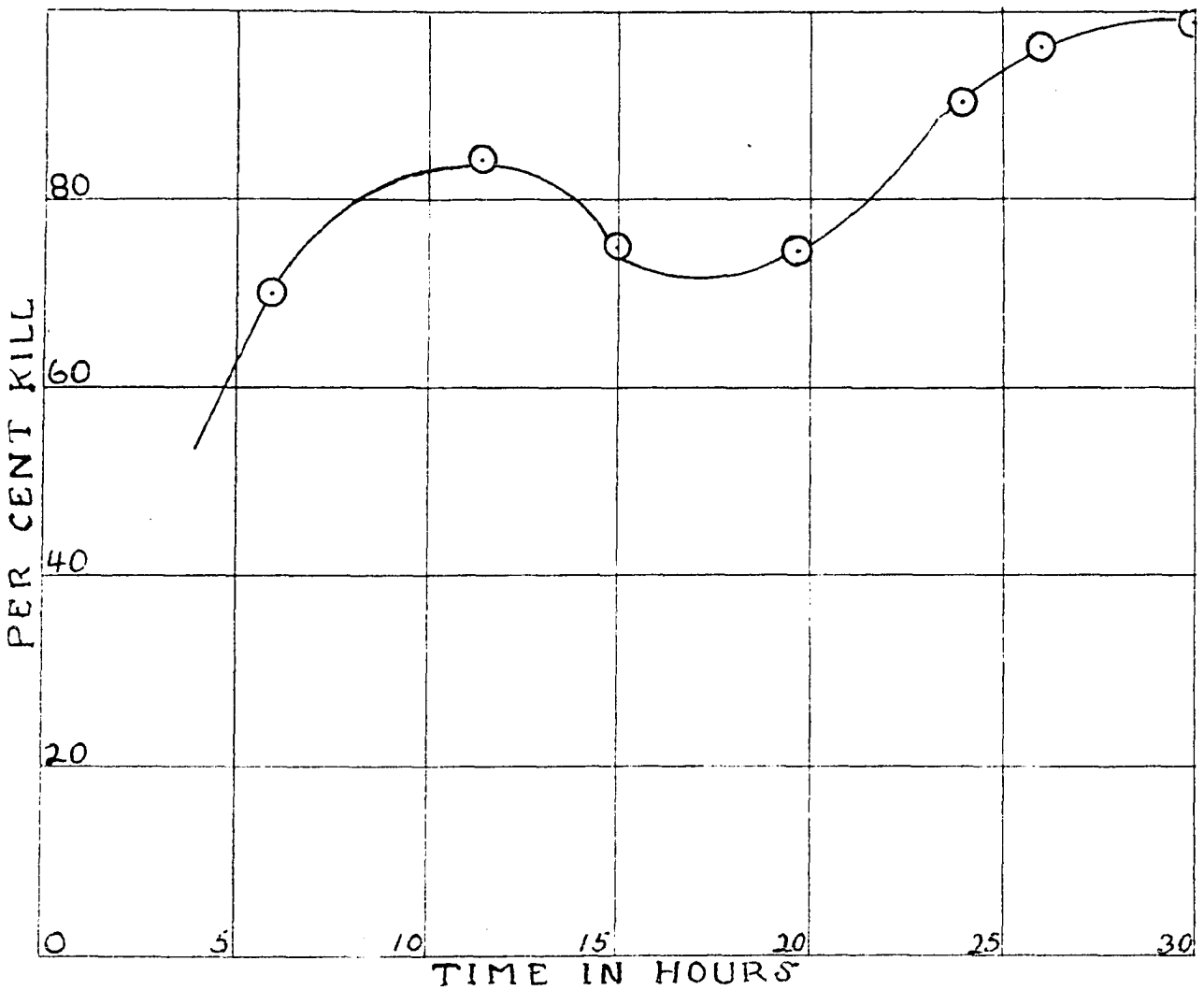

Fig. 10. A time course curve is drawn for weevils exposed to 381 parts of hydrocyanic acid gas per million parts of gas mixture. One hundred weevils were used to determine each point.

to produce one hundred percent kill at different concentrations. The results are summarized in the following table.

TABLE IV.

The Weevil		The Cockroach		The Rat	
Conc. of HCN in Parts per Million	Time in Min. for 100 per-cent kill	Conc. of HCN in Parts per Million	Time in Min. for 100 per-cent kill	Conc. of HCN in Parts per Million	Time in Min. for 100 per-cent kill
15,000	270	3523	25	783	4
12,900	270	2044	35	565	5
10,460	360	1630	40	532	7
9,000	420	1435	50	364	7
7,500	450	869	75	270	25
		689	105		
		407	180		

The time intervals necessary to produce one hundred per cent kill are plotted against the corresponding concentrations in Figure 11. These may be called lethal concentration time curves. Curve a is for the rat, b for the cockroach, and

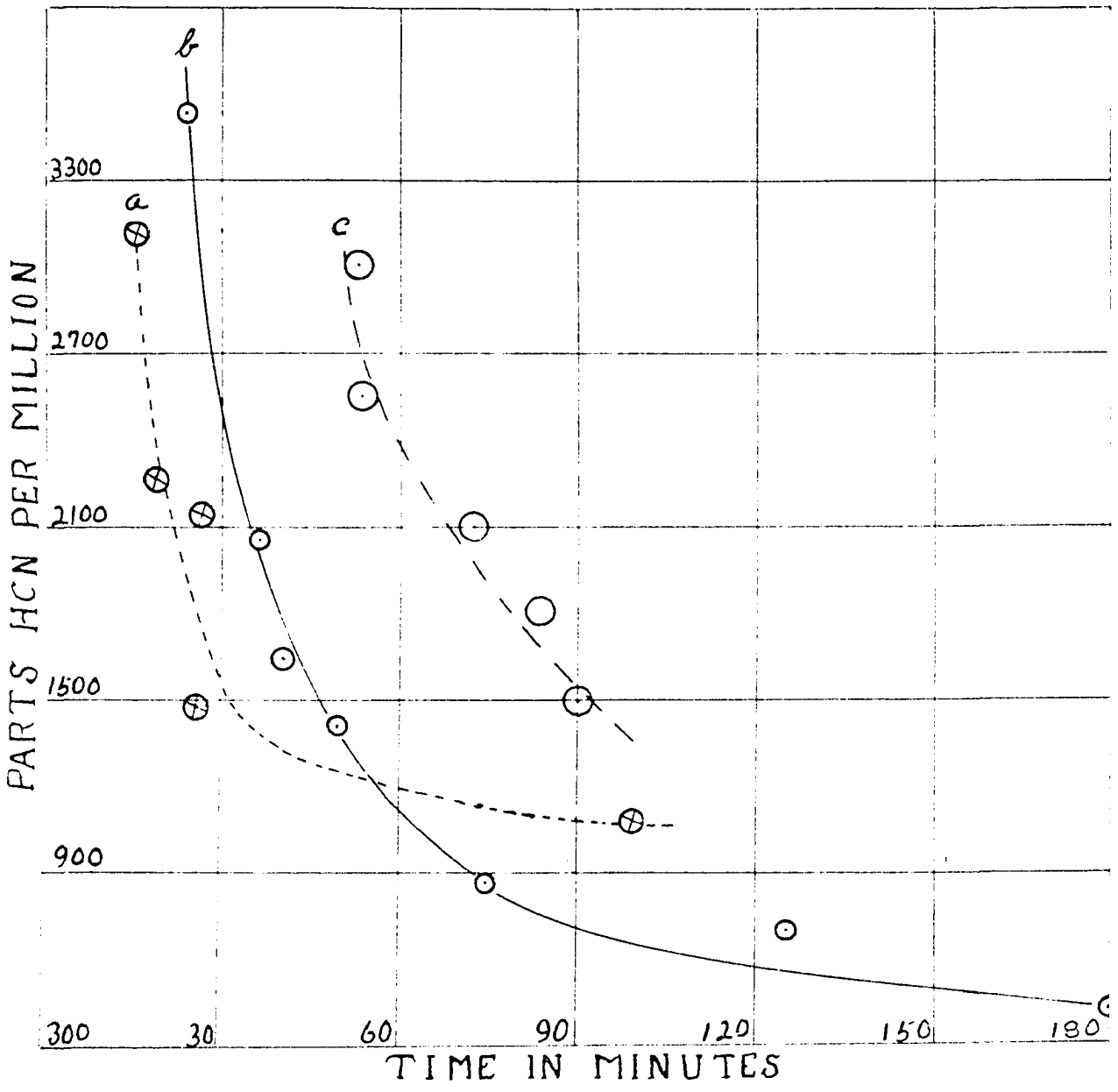

Fig. 11. Concentrations are plotted against the time interval necessary to produce 100% kill. Curve a is for the rat, b for the cockroach and c for the weevil. The cockroach curve is taken as the standard. Divide by four to obtain the correct values for the rat and multiply by five for the weevil.

c for the weevil. For ease of comparison, the cockroach curve b is taken as the standard and is plotted directly against the concentrations of hydrocyanic acid and time which are written on the chart. In order to obtain the true concentrations and time intervals for the weevil curve those written on the chart must be multiplied by five, and for the rat, they must be divided by four.

It is evident from a study of these curves that the rat is much more susceptible to the gas than is the cockroach and the cockroach in turn is much more susceptible than the weevil. The type of curve is also of interest. To quote from Safro (1927) "Dosages can generally be worked out for an entire range of exposures from minimum quantity of fumigant on the one extreme to minimum length of exposure on the other. The interest affected can then choose the dosage and exposure that more nearly meets its requirements. In ship and car fumigation the time element is usually of major importance and the shortest exposure would be preferred. In warehouse fumigation frequently the lowest dosage would be preferred, with overnight or week-end exposures." It is evident from the curve in Figure 11 that there is little practical value in using concentrations above five hundred parts per million for the rat and twenty-one hundred

parts for the cockroach.

It is true that in actual practice the dosage must be controlled by such factors as the affect on plants or seeds and the leakage of the gas from the space to be fumigated. There is also the factor of rate of distribution of the gas. The work of Komp (1921) and Knight (1925) would indicate that a uniform distribution of hydrocyanic acid is to be desired and Knight (1925) and Quayle (1927) have found that the use of calcium cyanide approaches the ideal. Other conditions such as relative humidity light, temperature, etc., have been carefully investigated for practical fumigation. It is believed that with the knowledge gained by field workers the curve obtained for an insect at ordinary temperatures would be of great assistance for the determination of the most practical concentration of hydrocyanic acid gas to use for its destruction within a definite time limit.

The shape of the curves would also help explain the fumigation constant (dosage X time = K) mentioned by Knight (1925). This constant can be approximately true over a limited range which depends upon the course of the curve. Another conclusion which can be drawn is that it would be difficult to determine the minimum lethal dose unless there is an abrupt change in the slope so that the curve parallels the X axis. If it

gradually approaches the X axis it would be difficult to determine a true minimum lethal dose. Any comparison of fumigants made by the minimum lethal dose could not be accurate. The method of holding the time constant and then finding the concentration of different frumigants necessary to produce one hundred percent kill is also open to objection. There is no reason to believe that every fumigant would give the same type of curve, hence no comparison as to the rate of toxic action can be made. For example, one molar percentage of carbon disulphide and the same concentration of ethyl formate might give one hundred percent kill in twenty-four hours yet at higher concentrations the carbon disulphide may be much more efficient as an insecticide than ethyl formate.

Before ending the discussion of the data presented so far, it would be well to point out that there is also a relationship between the type of curve plotted in Figure 11 and the time course curve. The former curve is merely drawn through the one hundred percent kill points of the latter. Since the slope of the time course curve increases with the concentration, it will indicate the part of the other curve about which the experiment is being conducted.

Comparative Toxicity of Hydrocyanic Acid Gas Generated from Liquid Hydrocyanic Acid and from "Calcyanide".

In recent years the use of powdered calcium cyanide as an insecticide has been replacing the older methods of generating the gas from sodium cyanide or of using liquid hydrogen cyanide. It was soon found that the dosage scale for calcium cyanide in many cases was much less than when either of the former methods was used. To quote from Quayle (1927) who worked on citrus fumigation, "In the amount of calcium cyanide given there is about 1/4 less HCN than in the amount of liquid given." Three explanations for this apparent super toxicity can be offered. First, since the dust is blown into a tent in citrous fumigations, the close proximity of the dust particles may have an injurious affect. More hydrocyanic acid gas may be generated around the insect than would be calculated for the entire space. Second, there may be less leakage of the gas from the space fumigated when calcium cyanide is used than by the liquid or the sodium cyanide method. Third, the gas generated from calcium cyanide may be super toxic.

Quayle (1927) found that actually less gas does escape from a tent fumigation when "citro fume" dust is used than when the liquid is used. To explain the difference in the

ratio of the dosages given for the liquid acid and calcium cyanide, he states, "If the mean concentration of HCN gas under the tent is the same when these ratios are used then there must be less escape of gas in one case than the other. Where a gas tight fumigatorium is used for the comparisons the differential indicated does not appear. It would seem, then, that at least the second explanation is a valid one.

To determine whether or not the third explanation offered for the apparent supertoxicity of calcium cyanide is correct, a number of toxicity experiments were made using both the liquid and "Calcyanide" in the generating train. These experiments are summarized in the following table.

TABLE V.

ON THE COCKROACH

Conc. of Hydrocyanic Acid in Parts per Millions	Time in Minutes for 100% Kill	Gas Generated from
3523	25	"Calcyanide" (1)
1435	50	"Calcyanide" (1)
869	75	Liquid HCN
764	90	"Calcyanide" (2)
689	105	"Calcyanide" (3)
660	90	"Calcyanide" (1)
563	120	Liquid
505	120	"Calcyanide" (4)
407	180	Liquid

NOTE: The numbers after "Calcyanide" indicate the day it was used. For example if it was put into the generating train in the morning the number (1) means that the experiment recorded in the table was run during that day. The apparatus was kept going for four days without stopping and the numbers (2) (3) and (4) indicate the second, third, and fourth day that "Calcyanide" was used.

A glance at Table V shows that as the concentration of hydrocyanic acid gas decreases the time interval increases in every case except for six hundred and sixty parts per million. This is true no matter the source of gas or the number of days the "Calcyanide" has been in use. It will be found that these points will practically fit the curve in Figure 11. for the cockroach. Our only conclusion can be that there is little if any difference in toxicity between the gas generated from "Calcyanide" and from liquid hydrocyanic acid. It would appear then, that the smaller dosage required when using calcium cyanide is not due to a supertoxicity of the gas generated from it.

SUMMARY

A review of the literature on the toxicology of hydrocyanic acid and potassium cyanide is given.

A review and discussion is made of the literature on methods of illustrating and interpreting the action of poisons.

A description is given of an apparatus for generating hydrocyanic acid gas and for controlling its use in toxicity experiments.

The Francis and Connell (1913) colorimetric method for the analysis of hydrocyanic acid gas is adapted to this work.

The Liebig method of analysis for hydrocyanic acid is adapted to this work.

The experimental procedure is outlined and discussed.

The attempt is made to discuss the results in such a way that the value and relationship between the different methods of studying toxicity will be apparent.

The relative toxicity of gas generated from "Calcyanide" and from liquid hydrocyanic acid is determined and found identical.

CONCLUSIONS.

The modified Francis and Connell (1913) colorimetric method for the determination of small amounts of hydrocyanic acid gas will give accurate results.

The Liebig method for the determination of hydrocyanic acid is much simpler and more dependable than the colorimetric one. By using a micro burette concentrations as low as two hundred parts of the gas per million parts of air gas mixture can be determined with accuracy.

The time course curves, the mortality rate curves, and the lethal concentration time curves are all related and would be of value to the Economic Entomologist for determining the most efficient dosage of a fumigant.

The slope of the time course curve is increased with increased concentrations. Variations in the shape of the curve is probably due to a summation of all the factors which enter into the reaction of living tissue with its poisoned environment.

The shape of the lethal concentration time curve would indicate that a method of comparing fumigants by determining the minimum lethal dose could not be accurate.

A method of comparing fumigants by holding the time constand and then finding the different concentrations necessary

to produce one hundred percent kill can only be accurate at that particular time. The lethal concentration time curves for the different fumigants may not follow the same type of course.

The rat is much more susceptible to the toxic action of hydrocyanic acid gas than the cockroach and the cockroach in turn is much more susceptible than the weevil.

There is no difference in the toxic action on cockroaches of gas generated from "Calcyanide" or from liquid hydrocyanic acid.

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