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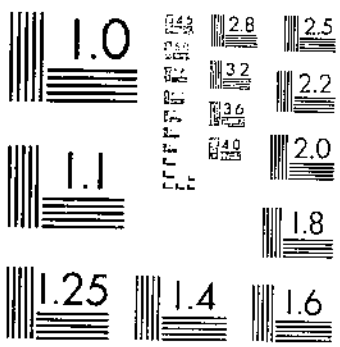

MICROCOPY RESOLUTION TEST CHART
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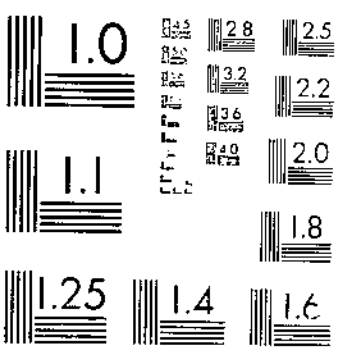

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TECHNICAL BULLETIN No. 346 MARCH, 1933

UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

THE EFFECT OF CONCENTRATION ON THE TOXICITY OF CHEMICALS TO LIVING ORGANISMS

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CONTENTS

INTRODUCTION

The determination of the toxic effect of different chemicals upon living organisms is of interest to various groups of investigators. Consequently, the literature contains much information on the toxicity of various materials to animals and to plants.

It is the purpose of this bulletin to present a relationship between the concentration of a poison and its physiological effect upon a wood-destroying fungus and to point out that there seems to be a definite connection between the constitution of the chemical and the rate at which the toxic effect changes with changes in concentration. The relationship between the concentration and the physiological effect of a poison also appears to be applicable to aphids, to bacteria, and to green plants.

[1] This bulletin embodies the results of toxicity investigations made by the Forest Products Laboratory over a period of 10 years. It has been the author's privilege to draw without restraint upon the accumulation of information that has resulted from studies of toxicity by other members of the laboratory staff. The author wishes to acknowledge his indebtedness for the toxicity data to C. J. Humphrey, R. M. Fleming, and C. Audrey Richards of the Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture and particularly to C. H. Henningsen and R. H. Baechler, both of whom worked under the direction of the author at the laboratory.

[2] Maintained by the U. S. Department of Agriculture at Madison, Wis., in cooperation with the University of Wisconsin.

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EFFECT OF THE CONCENTRATION OF CHEMICALS ON THE RETARDATION OF THE GROWTH OF THE WOOD-DESTROYING FUNGUS FOMES ANNOSUS

The work of other investigators on toxicity has well established the fact that as the concentration of a poison is increased, the effect on the organism in general becomes more marked (*1, 4*).[3]

This bulletin attempts to show that the changes in toxic effect produced by changes in concentration can be stated mathematically. The data upon which this bulletin is based were collected in an investigation on the toxicity of various chemicals used as wood preservatives. A knowledge of the amount of chemical required to kill the organism would probably be most desirable; however, in a relative way, knowing the concentration at which the chemical prevents the growth of fungus, or the total inhibition point, is almost equally useful.

METHOD OF CONDUCTING THE TESTS

PETRI-DISH METHOD

The first part of the experimental work reported followed very closely the method of testing developed by Humphrey and Fleming (*7*) of the Division of Forest Pathology, Bureau of Plant Industry, Department of Agriculture, and modified by C. Audrey Richards of the same bureau. Richards (*13, p. 131*) describes the method as follows:

> Measured quantities of the hot filtered medium [which consisted of 25 grams Trommers' malt extract, 15 grams of bacto-agar dissolved in 1,000 c c of water] are poured into small bottles. Into similar bottles are weighed or measured amounts of preservative necessary to give the desired concentration. This concentration is based on the actual weight of the preservative in the final medium-preservative mixture. The bottles containing the proper amounts of preservative and medium are sealed, clamped into frames, and sterilized. The preservative and medium are kept separate until after sterilization in order to reduce the possibilities for chemical combinations. After sterilization the medium is poured into the preservative bottle under sterile conditions and the contents thoroughly mixed. In the case of water-soluble substances the mixture is easily obtained by shaking by hand, but in the case of oils it is necessary to use a specially designed motor-driven shaker. Just before the mixture reaches the temperature where the agar solidifies, it is poured into a sterile Petri-dish 100 mm in diameter and 15 mm deep. In using oily preservatives, when emulsions—not solutions—are obtained by the shaking process, the medium-preservative mixture is hardened immediately by placing the Petri-dish on ice. This quick cooling insures a uniform, finely divided emulsion.
>
> After the medium has cooled, a small piece of fungus cut from a vigorously growing Petri-dish culture is planted in the center of each dish. The inoculated dishes are then placed in an incubator and held at a constant temperature for from four to six weeks. Frequent growth readings are made.

The fungus used in the present work was a strain of *Fomes annosus* developed at the Division of Forest Pathology. It is exceptionally well fitted for this work because it is very resistant to the toxic action of chemicals, and it normally produces an easily measured growth in cultures. This strain has been adopted by the Division of Forest Pathology and others as the standard fungus for all toxicity testing for wood preservatives.

Falck (*3*) has shown that when the fungi *Merulius silvester, M. domesticus, M. sclerotiorum, Polyporus vaporarius spumarius, Verpa bohemica, Phycomyces nitens,* and *Mucor mucedo* are grown in a nutrient

[3] Italic numbers in parentheses refer to Literature Cited, p. 52.

agar-agar medium at constant temperature, the length of the mycelium is directly proportional to the age of the culture so long as the food and air supply are sufficient and other conditions remain constant.

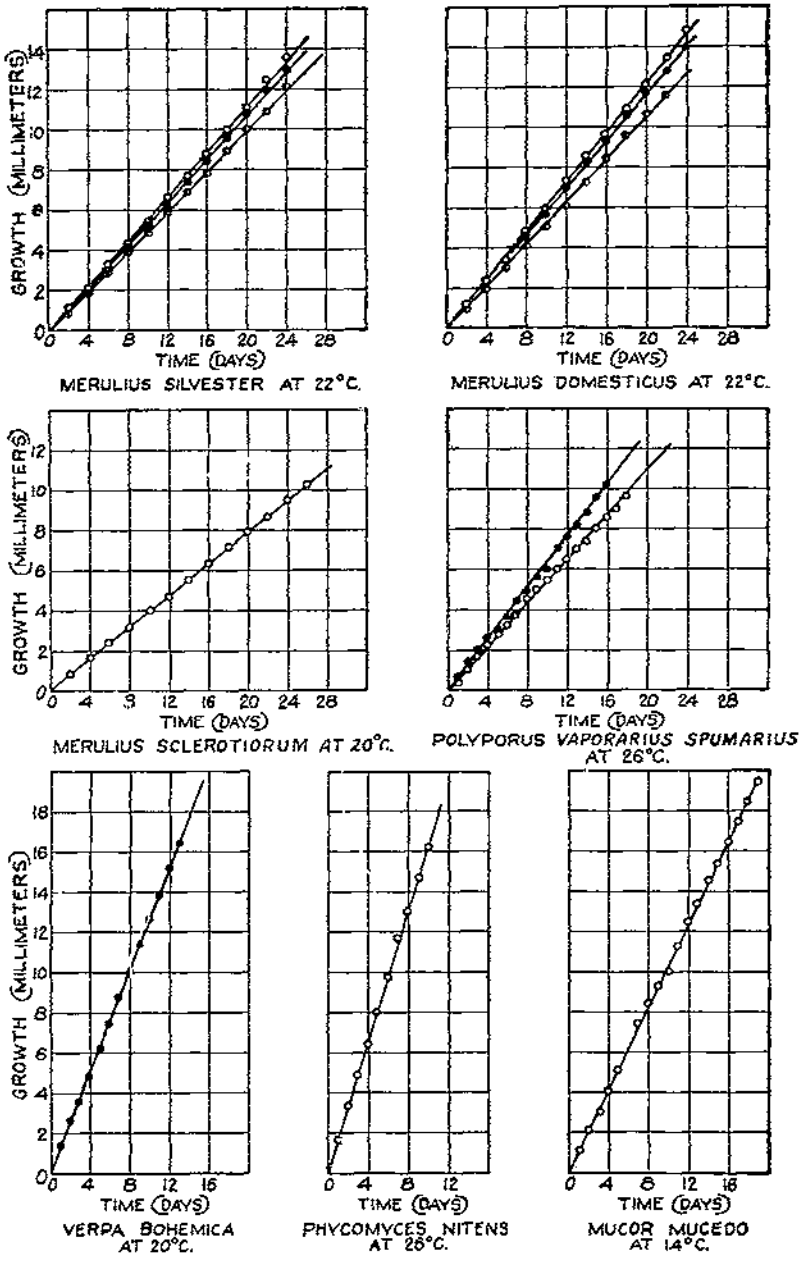


FIGURE 1.—Normal rate of growth of seven fungi. From data by Falck (3)

(Fig. 1.) The fungus *Fomes annosus* acts the same in this respect as those used by Falck. Furthermore, if the fungus is grown on an agar-agar medium containing a small quantity of a poison, the radial

growth of the fungus on a Petri dish will still be directly proportional to the time of growth, if the concentration of the poison is kept constant, although the rate of growth will be retarded. Figure 2 shows a number of straight-line relations when the fungus *F. annosus* is grown on a medium containing inhibiting material.

ERRORS IN THE PETRI-DISH METHOD

When the work was started it was not realized that the Petri-dish method gave rise to several errors in measurement which made some of the data of little value. This was particularly true of data collected over long periods of time. The existence of these errors was very forcibly shown in a matter of routine testing. In order that a large number of tests might be carried out as rapidly as possible, 75 or 100 Petri dishes with various amounts of coal-tar creosote were placed in the incubator at one time. At the time of the first reading, which was then one week after planting, the six or more control dishes that were always run with such a large number of dishes showed but little if any signs of growth.

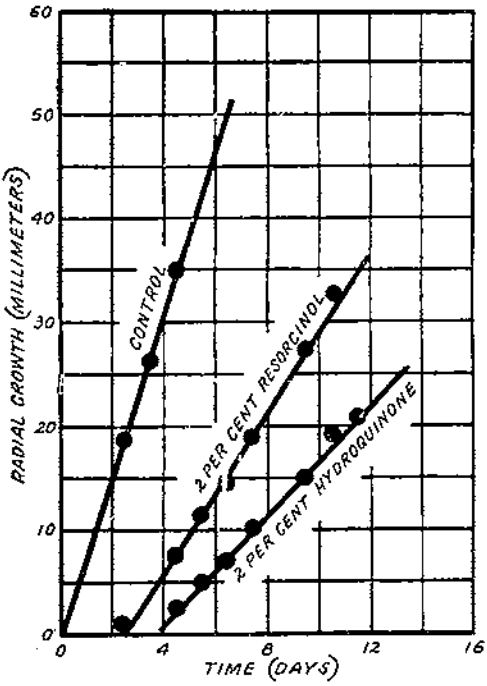


FIGURE 2.—Rate of growth of *Fomes annosus* on a nonpoisonous and on two poisonous mediums

The air in the incubator had become filled with the odor of coal-tar creosote, and it was therefore believed that the volatile portions of the oil had evaporated from the covered Petri dishes containing the creosote, and had diffused into the covered Petri dishes containing the control cultures, thus inhibiting growth in the control dishes. The large number of dishes under test made the amount of material that might evaporate in this way sufficient to almost saturate the air in the entire incubator. This explanation was valid only if it could be shown that the toxic effect of a chemical could be transferred by the medium of the air. Actual proof was obtained in the following manner:

A 2-liter glass-stoppered Erlenmeyer flask was used as a culture flask. A number of indentations were made in the neck to support a hollow glass bulb from whose lower extremity was hung a small glass dish or watch crystal, and the upper portion of the bulb was left open but was entirely contained within the flask below the ground-glass stopper. Several such flasks were then sterilized, and approximately 150 g of sterile nutrient agar was placed within each. The agar was planted in the usual manner when it had cooled and solidified. Imme-

diately after planting, various toxic materials, such as phenol, cresol, naphthalene, and benzene, were placed on the watch glasses suspended at the top of the several flasks; none of the toxic materials could have reached the agar except through volatilization. In every case the predicted toxic effect was obtained. Furthermore, it was possible to obtain only partial retarding by using less than the quantity required to kill. These retarded growths differed in no way from the retarded growth obtained from nonvolatile compounds in solutions in agar.

Though the foregoing experiment showed that the closed Petri dish was not a suitable container for testing volatile compounds, it also pointed out a possibility of error with nonvolatile compounds. The medium in which all of the tests were made was chiefly water, a volatile material. Losses of the water would change the concentration of the toxic material and hence the rate of growth. Consequently the resulting growth plots, if taken over periods of from four to six weeks, should be curves and not straight lines. Moreover, the concentration at the end of the test might be different from the concentration upon which the test was started. An examination of the data available at that time showed that many of the plots were obtained over long periods of time; with nonvolatile compounds the curve was less steep at the end of the test than at the beginning, but with volatile compounds the reverse was true. Both facts are in conformity with the previous discussion. In a closed flask, however, the time-growth curves are practically straight lines within the experimental error of measurement. This is shown by Figure 3, which represents the data obtained simultaneously with a closed glass-stoppered flask and with a so-called closed Petri dish.

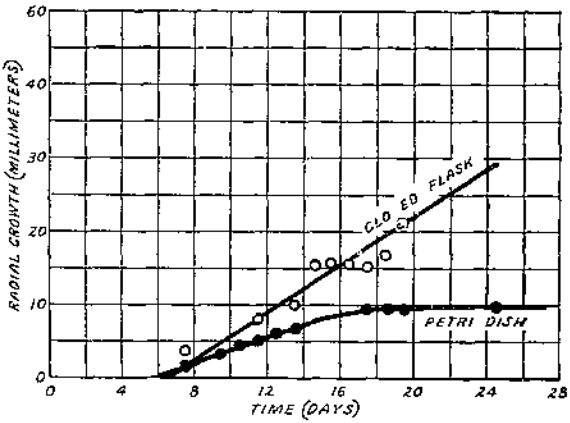


FIGURE 3.—Comparison between rate of growth of *Fomes annosus* on a medium containing hydroquinone solution in a closed flask and in a closed Petri dish

The loss of water from Petri dishes under test was of such vital importance that an attempt was made to gain a rough approximation of the amount lost. Three Petri dishes chosen at random were tared and the weights recorded after approximately 17 c c of nutrient agar had been added. These dishes were then placed in an incubator along with other material and weighed at the end of 11 and 24 days, respectively. The original weights and the losses are given in Table 1.

TABLE 1.—*Loss of water by evaporation from closed Petri dishes*

[Incubated at approximately 25° C.]

Original weight of agar	Loss at end of 11 days		Loss at end of 24 days	
Grams	*Grams*	*Per cent*	*Grams*	*Per cent*
17.85	5.62	32	11.12	62
18.03	2.32	13	6.16	34
18.55	6.94	37	11.71	63

There was considerable variation in the amount of water lost through evaporation, depending probably upon the tightness of the covers, but even the best result showed a loss in weight of 13 per cent in 11 days. Even with nonvolatile compounds an error of at least 10 per cent in the concentration would therefore be obtained at the end of 11 days. This loss was considered entirely too great for any but approximate determinations.

It was realized that with a more nearly airtight incubator the losses could have been greatly reduced, particularly if care had been taken to humidify the air in the incubator by the use of a relatively large dish containing water, but even then the Petri dishes could not be used with safety in working with volatile compounds. The Petri dish method was therefore discarded and a closed flask adopted instead.

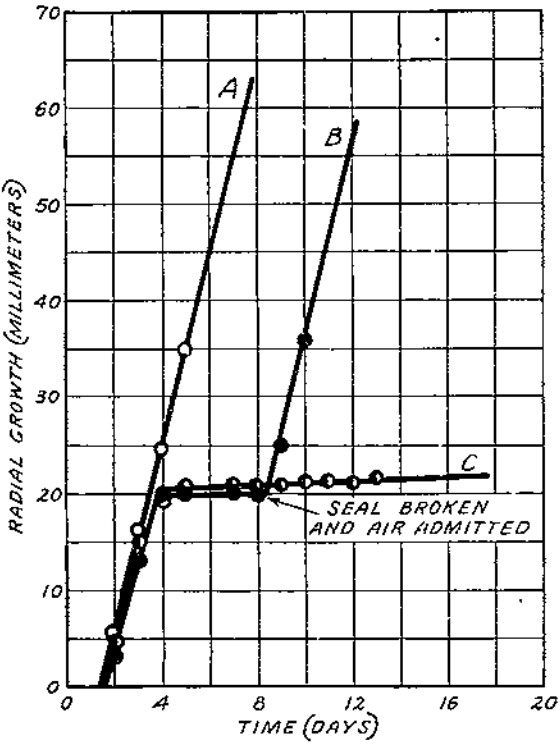


FIGURE 4.--Effect of lack of air on the rate of growth of *Fomes annosus*: A, Growth in unsealed Petri dish; B, growth in dish before and after breaking air seal; C, growth in covered dish with air-seal maintained

Perhaps the most important criticism that could be made of the use of a closed flask is that there might not be sufficient air to permit a steady normal growth of the organism. A few experiments were therefore made to determine the air requirement for the fungus.

The first of these consisted in selecting seven shallow Petri dishes of approximately 50 c c capacity. These were set with 17 c c of nutrient agar and planted in the usual way, but were sealed air-tight before being incubated. They were then incubated with three unsealed controls. Daily growth measurements were made on all the dishes at the same time. The results of three of these tests are shown diagrammatically in Figure 4; they show that air is an essential factor in toxicity testing. The largest Petri dish had a total capacity of 52.4 c c; subtracting from this the volume of the agar, or 17 c c, gives a total of 35 c c of air as being sufficient for only from two to three days' growth. One of the dishes was opened to determine if the rate of growth would remain the same after being retarded by lack of air. Curve B in Figure 4 shows that on the entrance of a new supply of air the rate of growth returned to normal.

The second experiment was carried out in large glass-stoppered, flat-bottomed weighing bottles having a total capacity of about 115 to 126 c c. The total air space in these bottles was reduced about 17 c c when the agar was inserted. The stoppers were sealed in, air-tight, after the agar had been inoculated. In every sealed bottle the growth at the end of three and one-half days exceeded the growth on the controls and had reached the edge of the dish so that no further measurements could be made. A maximum radius of 26 mm was obtained. On the supposition that the amount of air required is proportional to the area of the growth, it would appear that 400 c c of air should be more than is required for a growth equal to the radius of the flask. The ordinary wide-mouthed, 500 c c, glass-stoppered Erlenmeyer flasks fulfill the air requirement when 100 c c of agar are used. Flasks of this capacity or larger were used in the experiments here reported.

Some thought was given to replacing the glass-stoppered flasks by ordinary Erlenmeyer flasks with cork or rubber stoppers. It was shown that cork stoppers, even when covered with tin foil, were difficult both to sterilize and to keep in place without tying down. Rubber stoppers can not be used with volatile, oily compounds because of the absorption of the toxic material by the rubber. Moreover, they become hard with continued sterilization and nearly useless as stoppers after a few sterilizations.

FLASK METHOD OF TESTING TOXICITY

The method of testing finally adopted differed from the Petri-dish method only in replacing the Petri dish with glass-stoppered Erlenmeyer flasks with a minimum capacity of 500 c c. The quantity of agar used varied to some extent with the quantity of toxic material to be used; also with the size of flask, sufficient being used to insure nearly the same depth of agar in the center of the flask as was used in the Petri dishes. The detailed procedure was as follows.

The culture medium was prepared by heating with steam at atmospheric pressure for two hours a mixture of 1 liter of water, 15 g of Bacto agar, and 25 g of Trommer's malt sirup. After this mixture had been cooked it was filtered through cotton and measured quantities were poured into glass-stoppered Erlenmeyer flasks of 500 c c capacity. Usually 100 c c of agar was used in each flask. The quantity of agar was decreased when the material to be tested was available only in small quantities. When very low concentrations of a toxic material were to be tested the quantity of agar was increased; thus by increasing proportionately the quantity of toxic material used, the error of measurement was decreased.

The flasks containing the nutrient agar were stoppered with cotton plugs and the glass stoppers were covered with small pieces of cloth and fastened to the flasks during sterilization. The flasks were sterilized in an autoclave for 30 minutes at 15 pounds pressure. After sterilization a measured quantity of the toxic material was introduced either in the form of powder, liquid, or concentrated aqueous solution, depending on the nature of the toxic material. In many cases, particularly with sodium benzoate, it was impossible to introduce the toxic material as weighed powder because of contamination; a concentrated solution was found to be sterile. After the introduction of

the toxic material the glass stoppers were removed from their sterile cloth containers and placed in the flasks. The agar was then gently melted and the toxic material thoroughly incorporated by shaking. It was sometimes necessary to let the sterile agar stand two or three days to permit all parts of the agar to arrive at the same concentration; usually, however, the flasks stood overnight and were planted the next morning. The transplants, which were about 5 to 8 mm square, were taken from a culture of *Fomes annosus* that had been growing for from 5 to 12 days.

The concentrations used were recorded in percentage of the volume of agar taken because it was more convenient experimentally. When 100 c c of agar was taken, 1 g of toxic material equaled 1 per cent concentration, 1 mg of toxic material equaled 0.001 per cent concentration. For comparative purposes the molar basis is a better one to use. The experimental data have therefore been calculated to moles per liter by dividing the percentage concentration multiplied by 10 by the molecular weight of the compound under test.

DIFFICULTIES ENCOUNTERED WITH THE FLASK METHOD

While there are several minor difficulties, such as the darkening of the agar due to the interaction of the toxic materials and the fungus and the production of halos immediately beyond the growing fungus, only two seem worthy of discussion here and these only because they affect the values of the measurements. One of these difficulties was connected with the air requirement and occurred only in those flasks that contained easily oxidized materials, such as aniline or pyrogallol. In Figure 5 is a curve showing the growth on a solution of aniline. After nine days the growth of the fungus practically ceased, and no growth took place for nine days. A new supply of air was then admitted, and the growth started again at the original rate. The shape of the curve obtained here is almost identical with that shown in Figure 4, B, which was known to have been influenced by lack of air. In calculating the retarding effect on such curves the period of no growth was neglected.

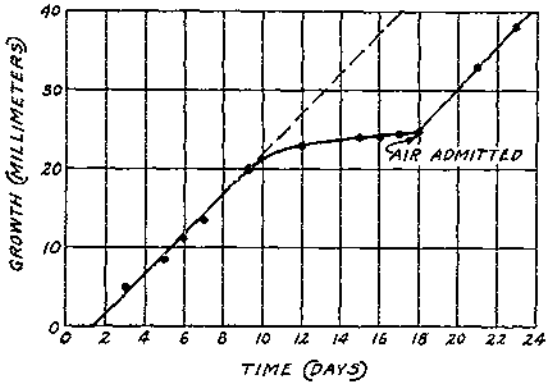


FIGURE 5.—Growth curves for *Fomes annosus* on a medium containing aniline solution showing rest period brought about by insufficiency of air

The other difficulty was encountered chiefly with oily toxic materials, such as benzene, chlorobenzene, or nitro compounds. With such materials, the surface of the transplant appeared at first to be of a gray slimy consistency; later this changed and the surface appeared like gray pebbly oilcloth. This condition often continued to the end of the test, but the transplant was not dead, because it recovered almost immediately on being transplanted to fresh, nontoxic agar.

Occasionally the transplant started to grow after long periods of no growth, and the growth continued at the same rate as that of a normal control culture. This rapid growth was even detected after 100 days of apparent inactivity. Under the microscope the fungous hyphæ appeared to divide into many smaller cells, each forming a chlamydospore. A few of these grew at the same rate as would be expected of the control. In one case, because a drop of condensed water ran down the side of the flask and over the transplant during the periodical examination, one of these sporelike bodies was carried away from the transplant so that it was possible to measure both the retarded growth

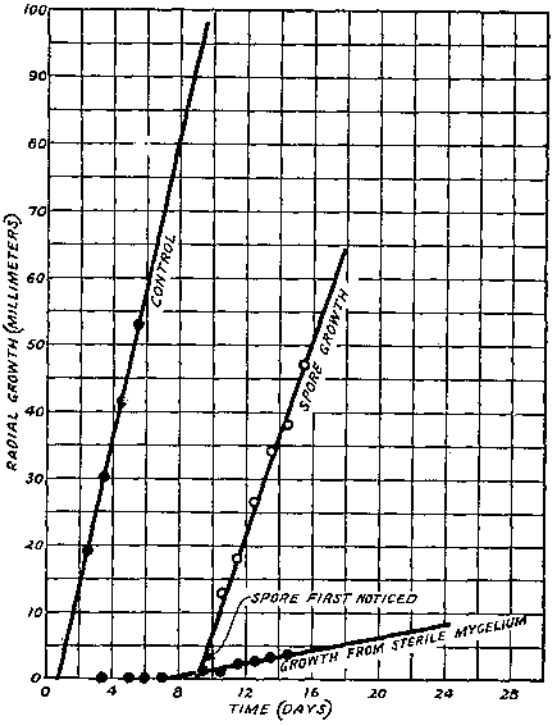


FIGURE 6.—Comparison of the rate of growth of a control with that of transplants originating from mycelium and from a spore

of the fungus and the rapid growth of the spore. These measurements are shown in Figure 6. In another case it was possible to detect the beginning of a spore growth by its luxuriant growth in the shape of a small pompom. Measurements were taken on both the retarded growth and the spore growth until the more rapid spore growth had entirely covered the slower retarded growth. These data are shown in Figure 7. If the two different growths had not been recorded in Figure 7, the curve would have resembled a steep curve such as might have been obtained if the loss of toxic material had been complete. While this apparent sporulation is of interest to the pathologist because it offers a possible explanation of the mechanism of accommodation, it is also of interest in the practical field of wood preservation

because it suggests that certain preservatives may appear to retard the growth, but if the organism is not killed there is always a chance of the formation of chlamydospores. It is evident that with such materials a different organism from the test organism, or at least a very different strain, must be considered. Whenever the growth was almost as rapid as the control, particularly if it followed a long inactive period, it was considered that a spore growth was responsible, and the particular test was repeated.

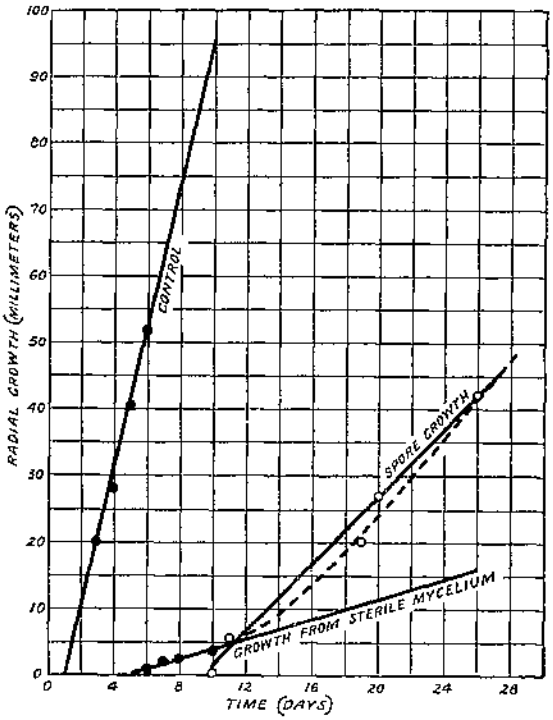


FIGURE 7.—Comparison of the rate of growth of a control with that of transplants originating from mycelium and from a spore. A combination of the two cultures yields a curve as shown by the broken line

CALCULATION OF EXPERIMENTAL DATA

When the radial growth of the fungus grown in closed flasks is plotted against the time of growth, a straight line is obtained at least within the limits of the radius of the containing flasks. Moreover, when the fungus is grown on agar containing a toxic material that retards the growth, straight lines representing time-growth curves are still obtained. This fact is illustrated in Figure 8. The chemicals represented in Figure 8, the exact composition of which is immaterial, have been introduced merely to illustrate the wide application of the straight-line relationship between growth and time when the cultures are grown in closed flasks.

The data obtained from the daily measurements of fungous growth are recorded in Table 2 and were later plotted on ordinary coordinate

paper along with the control. An almost ideal plot is shown in Figure 9 since all the growth lines appear to start at about the same time, but the growth is much slower than the control.

TABLE 2.—*Sample sheet showing method of recording data on the radial growth of Fomes annosus on a medium containing various concentrations of poison*

Time	Radial growth of *Fomes annosus* in a concentration of—						
	0 per cent [1]	0.02 per cent	0.03 per cent	0.04 per cent	0.05 per cent	0.06 per cent	0.07 per cent
Days	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Millimeters*	*Milli-meters*	*Milli-meters*
1							
2		2-2-2-2	1-1-1-1				
3	22-22-23-23			3-3-3-5	1-1-1-1	(2)	(3)
4	34-35-35-35	16-17-17-17	16-12-13-12			(2)	(3)
5	47-47-47-48			9-9-8-9	2-2-2-2	(2)	(3)
6	Covered.	28-29-29-29	17-18-17-17				
7				15-17-16-16	7-7-6-6		
8		Covered.	20-20-21-20	19-18-18-16	6-9-8-7	(2)	(3)
9				17-17-17-17	7-8-9-8		
10				20-22-20-23	10-11-11-10	(2)	(3)
11			32-32-33-32				
12				27-25-26-26	11-12-12-12		
13			36-38-38-30		11-15-13-12	(2)	(3)
14				30-30-30-30	15-15-15-15		
15						1-1-1-1	(3)
17					16-17-17-16	1-1-1-1	(3)
24						2-2-2-2	(3)
26						2-3-4-3	(3)
28						3-3-3-3	(3)
30							

[1] Control.
[2] Growth on transplant.
[3] Transplant white, no visible growth but no change to a slimy condition.

If the line in Figure 9 representing the growth of the control be extended until it reaches 100-mm radius and all the lines representing the retarded growth caused by the toxic action of the chemicals under test be read at this point on the time ordinate, the percentage of retarded growth is obtained without calculation. Subtracting the percentage of retarded growth from 100 per cent gives the percentage of retarding effect caused by the toxic action of the chemical. Either the percentage growth or the percentage of retardation provide a measure of the effect of the toxic chemical. The percentage of retardation gives values that are direct functions of the concentration and are easier to handle than the inverse functions of percentage growth. Throughout this work the percentage retardation has been used. The difference between the two systems of measurement is shown diagrammatically in Figure 10.

All of the data were not so ideal as that shown in Figure 9; in fact, the ideal condition of growth was obtained in only an occasional test. More commonly the retarded growths on the toxic solutions did not start immediately, because there was an initial rest period during which no growth was apparent. Even the control commonly took a day or more to get started. In some exceptional cases no growth was obtained on the control agar after two days and only slight growth at the end of three days, yet when the growth became established on the agar it continued as rapidly as if there had been no rest period.

It seems reasonable that the transplanting of agar containing the growing fungus from one position to another should cause a setback in the growth of the fungus. It also seems reasonable that if the

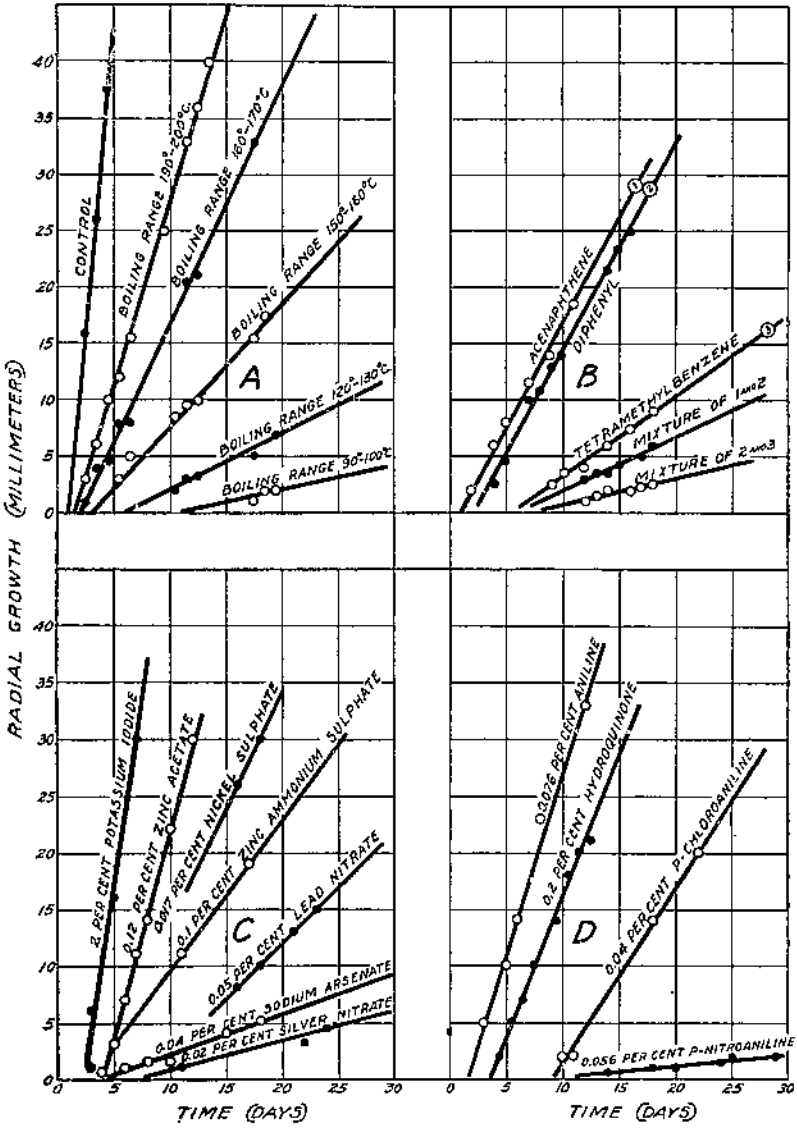


FIGURE 8.—Time-growth curves showing straight-line relation when *Fomes annosus* is grown on a medium containing toxic materials: A, Medium saturated with petroleum fractions; B, medium saturated with aromatic hydrocarbons; C, medium containing inorganic salts; D, medium containing organic compounds

transplant were placed on a medium on which it would have difficulty in maintaining itself that it would take a little longer time to become established. It might be suggested reasonably that this period of

delay in starting is a measure of the toxic effect of the medium upon which the fungus is trying to establish itself. If so, it is a very unreliable measure because the starting time varied over wide ranges even with the same concentration of the same toxic material. As shown in Figure 11, which diagrammatically presents the data for two identical concentrations of chloroaniline that were started at different periods of time, the starting time for one was 3 days and for the other 10 days. The controls for the two were duplicated, both as to starting time and rate of growth. For the present, therefore, the starting time must be looked upon, not as a measure of toxic effect, but as a rest or recovery period. The rate of growth on the toxic medium is a better measure of toxic effect. In Figure 11 the two lines representing the growth of the same concentration of chloroaniline but with different starting times, are parallel, indicating that the rate of growth is not affected by the rest period.

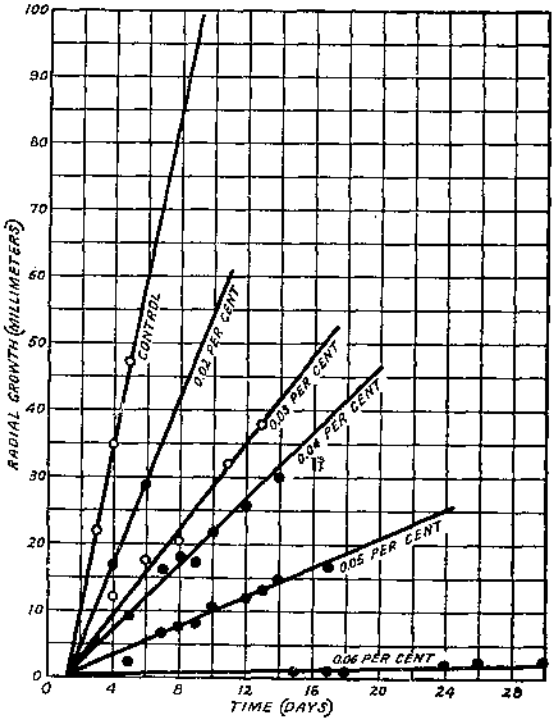


FIGURE 9.—Rate of growth of *Fomes annosus* on a medium containing different concentrations of *p*-nitroaniline

The simplest way of calculating the retarding effect, when the data contain rest periods, is to plot the data in the usual manner and obtain the best straight line through the data. Then draw a line parallel to this growth line in such a manner that the curve starts at the starting time for the control. From this corrected line the percentage retardation can be calculated in exactly the same way as it would be had the growth been ideal. Figure 12 shows the data collected on three concentrations of nitrobenzene and also the dotted lines which correct the data to ideal growth conditions. This method of plotting the data diminishes the experimental errors in two ways: (1) The plotting of the data to obtain the best straight line removes in large measure the errors caused by parallax and other difficulties in the actual measurement of the growth, and (2) the correction of this line to the ideal condition removes the error due to the resting period. The corrected data, therefore, give the most probable value for the percentage retardation and permit each concentration of toxic material to be represented by a single figure of the most probable percentage retardation.

The values obtained for the percentage retardation of the different concentrations of a single chemical were plotted against the various concentrations. In the resulting diagram the trend of the points may be along either a straight line or a curve, if ordinary coordinate paper is used, but conforms rather closely to a straight line on logarithmic paper if the logarithm of the percentage retardation is plotted against the logarithm of the concentration. The extension of the line to 100 per cent gives immediately the most probable total inhibition point, or the point at which no growth should be obtained. It also permits the data to be collected with a smaller number of tests, since each concentration serves as a check upon another concentration. The total inhibition point indicated by the extension of the line to 100 per cent can then be checked if more accurate figures are desired. For example, the data shown in Table 3 were obtained directly from the curves shown in Figure 9.

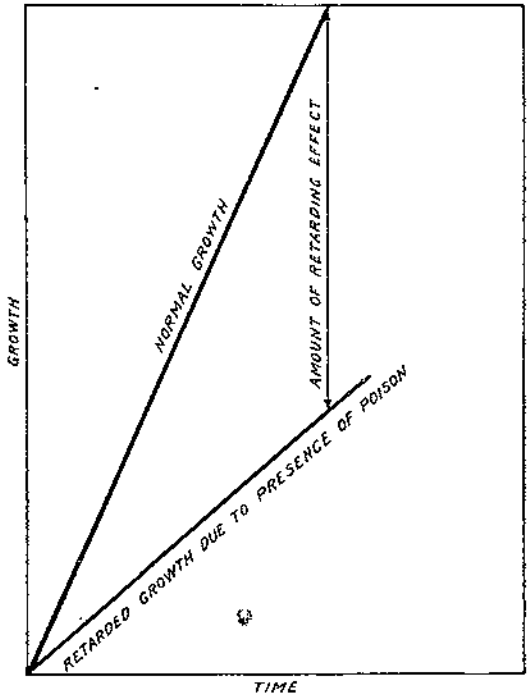


FIGURE 10.—Method of calculating percentage retardation

TABLE 3.—*Calculation of percentage of retardation for Fomes annosus on a medium containing p-nitroaniline solution*

[Data from the curves in Figure 9]

Concentration of *p*-nitroaniline	Growth obtained during same interval of time as was required for control to grow 100 mm in radius		Percentage retardation (100 per cent—per cent of retarded growth)	Concentration of *p*-nitroaniline	Growth obtained during same interval of time as was required for control to grow 100 mm in radius		Percentage retardation (100 per cent—per cent of retarded growth)
Per cent	*Mm*	*Per cent*	*Per cent*	*Per cent*	*Mm*	*Per cent*	*Per cent*
0.02	50.0	50.0	50.0	0.05	9.0	9.0	91.0
.03	26.0	26.0	74.0	.06	.7	.7	99.3
.04	19.5	19.5	80.5				

When the data in the first column were plotted on logarithmic paper against the data in the last column a straight line was drawn as shown in Figure 13.

DISCUSSION ON METHOD OF CALCULATING DATA

In general, the scheme of analysis and presentation of toxicity data used here depends upon two methods of plotting the results. These methods are (1) the plotting of the growth with time in such a manner that the effect of the different concentrations below the total inhibition point can be recorded in a numerical manner, and (2) the plotting of the numerical values for all the concentrations in such a manner that a straight line is obtained. The main objection to the method is that in general more labor is involved for an equal number of tests because greater attention must be given to those tests which are usually considered as negative. Aside from the fact that the data obtained seem to be of a more fundamental character, there are several other advantages in the scheme of analysis used here.

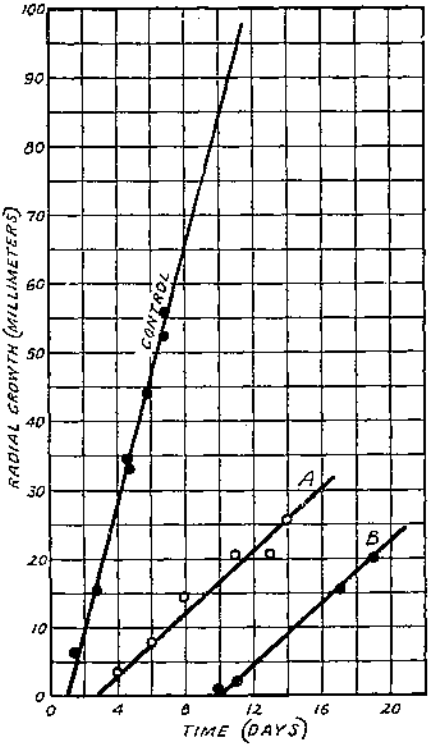


FIGURE 11.—Rate of growth of *Fomes annosus* on a medium containing p-chloroaniline solution of the same concentrations: A, Started growing 3 days after transplanting; B, started growing 10 days after transplanting

(1) A more effective control of the experimental conditions is made possible while the experiments are in progress, because experience has shown that such major variations from straight-line growth curves as are shown in Figure 5 are due to changes in concentration of the toxic material; these occur either because of evaporation of the water or the chemical itself, or because of the chemical action of the toxic material. In many such cases the concentration after a period of time is not the same as when the test was started. Experience has also shown that when curves are obtained which show little or no growth for quite a period, followed by a growth almost as rapid as that of the normal culture, it is almost always because of the production of spores from which, apparently, a more resistant strain of fungus is obtained.

(2) A smaller number of check tests are necessary because the method of plotting makes each concentration serve as a check on all other concentrations.

(3) Since the method requires the recording of the effect of concentration below the total inhibition point in a numerical manner, it permits the calculation of the effect of a combination of two or more toxic materials which by themselves are insufficiently soluble totally to inhibit growth. This use is best illustrated by Figure 8, B, which shows the growth curves for saturated solutions of diphenyl

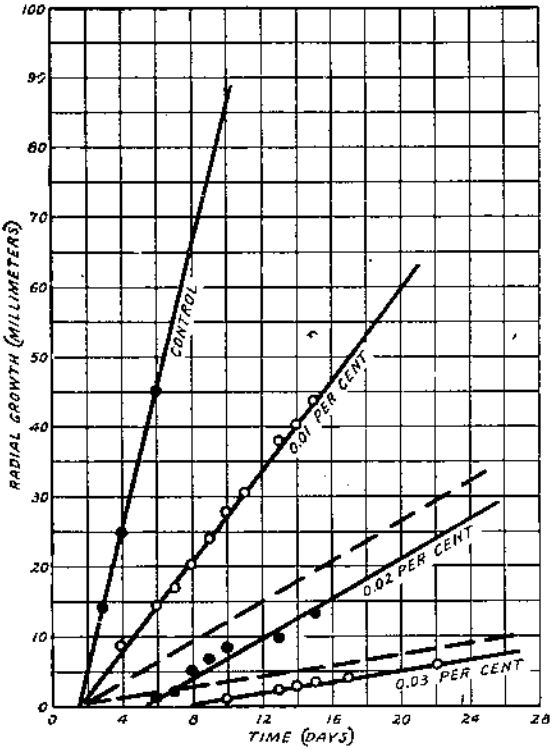


FIGURE 12.—Rate of growth of *Fomes annosus* on mediums containing different concentrations of nitrobenzene. Solid line represents experimental data; broken line represents data corrected for rest periods

and acenaphthene and for a saturated mixture of the two in the same test. The percentage retardation for acenaphthene as given in the footnote to Table 4 is 82.6 per cent; for diphenyl 84 per cent. If the two compounds reacted independently of each other, it would be expected that the growth permitted under their combined effect would be the product of the respective retarded growths produced by their use, because the retarded growth with the first chemical becomes the equivalent of the normal growth as far as the second chemical is concerned. Therefore, the combined effect should be 0.174×0.160, or 0.028, or 2.8 per cent, or a retardation of 97.2 per cent. Two other pairs of compounds, namely, durene and diphenyl, and durene and acenaphthene, gave substantially the same kind of results, as is shown in Table 4. The agreement between the experimental results and those obtained by calculation seems to be somewhat better than fortuitous. If this is the proper way of calculating the effect of saturated solutions of more than one compound, none of which is alone able completely to inhibit the growth of the fungus, this leads to the conclusion that, from a theoretical point of view, no combination of such compounds would ever be able completely to inhibit the growth of the fungus, although it might very

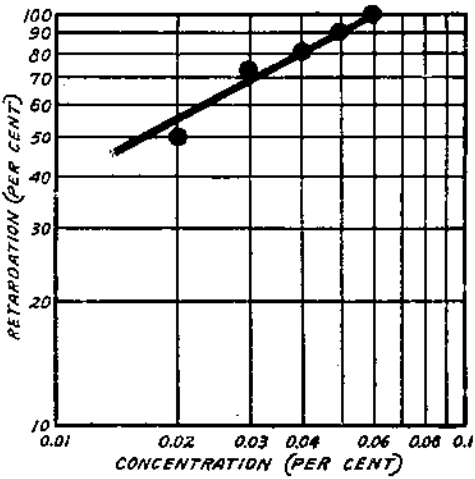


FIGURE 13.—Example of plotting percentage retardation against concentration

seriously retard it. This idea was tested by combining diphenyl, durene, and acenaphthene. The combined calculated effect should be a percentage retardation of 99.88 per cent, or a growth of 1½ mm in 100 days. At the end of 21 days no visible growth was noticed, but the fungus was not dead, because by merely aerating the flask to remove the hydrocarbon the vitality of the fungus was restored, and at the end of the third day after aeration started it was apparently making the same rate of growth as that on the unpoisoned medium. These calculations make it appear probable that when saturated solutions of materials, none of which is by itself soluble enough to exert a full lethal effect, are combined, the combined effect of two or more can be calculated from the individual results, provided the solubility of none of the materials has been affected by the presence of the others. The calculation raises a very interesting point as to what would be the effect of two or more soluble toxic materials administered in doses which, if each material were taken alone, would be insufficient to kill the organism. It also raises the question whether or not a similar mode of action may be expected in a solution containing toxic basic ions, toxic acid ions, and toxic undissociated molecules. Should such a relationship, or one based on similar principles, occur, it would aid materially in the work on mixed toxic materials.

TABLE 4.—*Comparison of calculated and experimental results when saturated solutions of two hydrocarbons, neither of which is lethal, are used*

Mixture contains—	Calculated retardation [1]	Observed retardation
	Per cent	*Per cent*
Acenapthene and diphenyl	97.2	96.6
Acenapthene and durene	99.2	98.8
Diphenyl and durene	99.3	99.4

[1] Percentage retardations of saturated solutions of each alone are: Acenapthene, 82.6 per cent; diphenyl 84 per cent; and durene, 95.6 per cent.

MATHEMATICAL DISCUSSION

In order that the succeeding discussion may be understood more easily, it seems desirable to give a short discussion at this point of the mathematics involved in the experimental work. Since it has been shown that within the limits of the experimental work the radial growth of the fungus can be represented by a straight line on ordinary plotting paper, the relation between radial growth and time can be expressed by the general equation of a straight line, or $G=K(t-m)$, in which G is the radial growth, K the slope of the line, t the time, and m the rest period that occurs before the fungus grows. The method of comparing the different rates of growth eliminates m as being of no consequence in this connection. The equation can therefore be written:

$$G=Kt \text{ or } K=\frac{G}{t}$$

where t is the time elapsing after the rate of growth has become established.

K the slope represents the rate of growth of the fungus in the particular experiment. In order to compare the rate of a retarded growth with the rate of the normal growth, the slope for the retarded growth has been expressed as a percentage of the slope for the normal growth; that is,

K retarded growth = K normal growth times the percentage of retarded growth

This procedure gives inverse functions of the concentration; that is, as the concentration increases, the values of K normal growth times the percentage of retarded growth decrease. This was obviated by subtracting the percentage growth from 100 and calling the result the percentage retardation, or R. The actual growth in millimeters per day on any toxic solution is therefore expressed as

$$K_{\text{normal growth}}\left(1.00-\frac{R}{100}\right)=\frac{G}{t}$$

When the values of R were plotted against the concentration on logarithmic paper the data conformed closely to a straight line; that is,

$$\log R=\log D+n \log C$$

in which R is percentage retardation, C the concentration of the toxic material, $\log D$ a constant, and n the slope of the line. The normal form of this equation is

$$R=DC^n$$

which is the general equation of a parabola.[4] The straight line shown in Figure 13 therefore represents a parabola, and the relation between percentage retardation and concentration is a parabolic relation. The values of C, the concentration, may be expressed in terms of percentage concentration or in terms of moles per liter without changing the value of the slope, but the value of the constant D will be changed by a value that depends on the n^{th} power of the ratio between the values in percentage concentration and moles per liter.

In the growth equation

$$K_{\text{normal growth}}\ (1.00-R)=\frac{1}{t}\ G$$

R can therefore be replaced by DC^n to form the equation

$$K_m\ (1.00-DC^n)=\frac{1}{t}\ G$$

When the values of K_m, the normal growth, and D, C, and n are known, the effect of any concentration within the limits of the parabolic relation can be calculated and should reproduce the actual experimental figures corrected for the rest period.

[4] Parabola or parabolic curve as used in this bulletin refers to any of the curves represented by the general equation $y=ax^n$, where n is positive, and not with the restricted meaning where $y=ax^2$.

EXPERIMENTAL RESULTS

When the percentage retardation for the various toxic materials was plotted against the concentration as given in the preceding discussion, the straight lines on logarithmic paper had a number of different slopes. It was first thought that the slopes of the various lines were of little or no consequence but that they were characteristic of the individual chemical. It was found, however, that as the data accumulated there were several groups whose slopes were nearly the same, that the average of these would portray the data fully as accurately as the experimental work justified, and that in each group there existed a similarity of chemical construction that did not seem to be entirely fortuitous. The six following figures show the concentration-retardation curves for these groups of chemicals. Within each group the line representing the change in percentage retardation with changes in concentration is parallel to the other lines in the same group and interprets the data within the limits of the experimental error of the methods used.

INORGANIC COMPOUNDS

The inorganic compounds naturally divide themselves into two groups: (1) Those chemicals whose basic radicals, such as silver, mercury, and copper, can be considered as the toxic radical, and (2) those compounds whose acid radicals, such as arsenate and tungstate, may be considered as the toxic radical. In general, the salts are preferred for this type of work because they eliminate the effect of the hydrogen or hydroxyl ions. Growth of the fungus *Fomes annosus* has been obtained on approximately half-molar solutions of sodium chloride, potassium chloride, magnesium sulphate, and calcium acetate; none of these radicals can be considered as very toxic.

Figure 14 gives the data collected from inorganic salts whose basic radicals may be considered as toxic, and Figure 15 gives the similar data collected from inorganic salts whose acid radicals may be considered as toxic. Tables 5 and 6 give the total inhibition points as determined by extending the percentage retardation-concentration curves to 100 per cent (p. 20).

TABLE 5.—*The most probable total inhibition points of salts in which the basic radical is poisonous*

Salt	Highest concentration at which fungus grew	Lowest concentration at which no growth was obtained	Most probable total inhibition point	Salt	Highest concentration at which fungus grew	Lowest concentration at which no growth was obtained	Most probable total inhibition point
	Mole per liter	*Mole per liter*	*Mole per liter*		*Mole per liter*	*Mole per liter*	*Mole per liter*
Nickel sulphate	0.00092	0.0015	0.00115	Silver nitrate	0.00132	0.00153	0.00137
Nickel ammonium sulphate	.0012	.00172	.00140	Copper chloride	.0017	.0023	.00182
Mercuric chloride	.00017	.00021	.00022	Uranium acetate	.0024	.0028	.00272
Thallous sulphate	.0004	.00058	.00047	Cadmium sulphate	.0057	.0056	.0066
				Zinc acetate	.011	.0165	.0143

TABLE 6.—*The most probable total inhibition points of salts in which the acid radical is poisonous*

Salt	Highest concentration at which fungus grew	Lowest concentration at which no growth was obtained	Most probable total inhibition point	Salt	Highest concentration at which fungus grew	Lowest concentration at which no growth was obtained	Most probable total inhibition point
	Mole per liter	*Mole per liter*	*Mole per liter*		*Mole per liter*	*Mole per liter*	*Mole per liter*
Sodium arsenite	0.002	0.0025	0.0025	Sodium tungstate	0.0235	0.067	0.067
Sodium arsenate	.0028	.0039	.0039	Ammonium molybdate	.33	.39	.39
Sodium chromate	.0013	.0030	.0015				

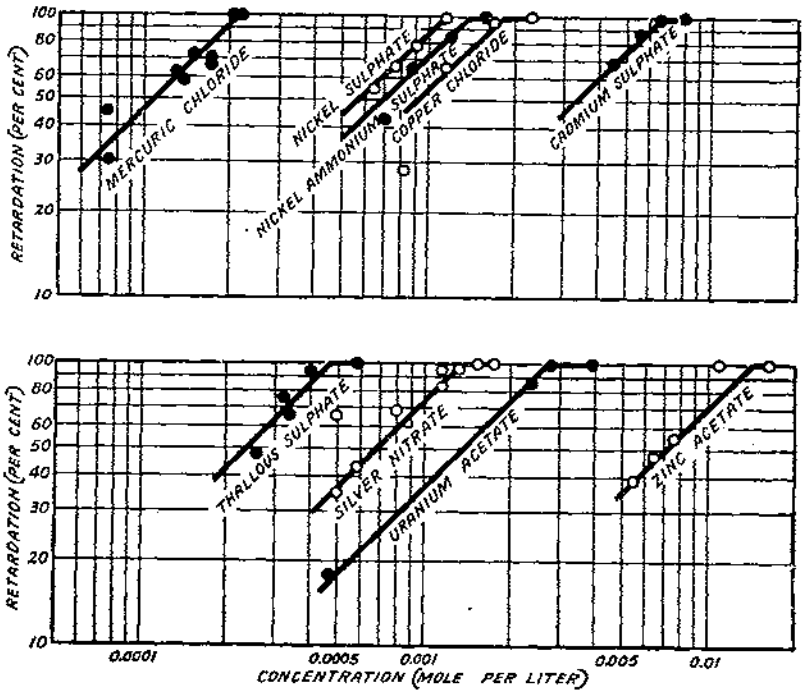


FIGURE 14.—Concentration-retardation curves for inorganic materials with poisonous basic radicals against the fungus *Fomes annosus*

It is evident that the slopes of lines given in Figures 14 and 15 are quite different. Lines with a slope that seems to fit the acid radicals can not be drawn through the points representing the data for basic radicals, or conversely.

ORGANIC CHEMICALS

Very little if any data are available on the toxicity of aliphatic compounds; most of the work has been confined to the aromatics, chiefly benzene derivatives. The benzene derivatives present a number of possible combinations having different structures. The disubstituted compounds are perhaps better suited to show the effect of differences in structure than any other class because many disubstituted compounds can be obtained that have the same substituent

groups in different positions. It would be expected that the greatest difference would be obtained by comparing the ortho with the para disubstituted compounds since these present the greatest structural differences.

Figure 16 presents the data obtained with para substituted benzene derivatives. Figure 17 gives similar data obtained with ortho substituted benzene derivatives. Tables 7 and 8 give the total inhibition points of these compounds as determined graphically. The lines representing the data for the ortho compounds can not be made to represent the para compounds, or conversely. This difference in behavior can only be explained by the difference in structure since the two groups of compounds contain the following structural isomers: *p*-chlorophenol, *p*-aminophenol, *p*-nitrophenol, *p*-chloroaniline, *o*-chlorophenol, *o*-aminophenol, *o*-nitrophenol, and *o*-chloroaniline.

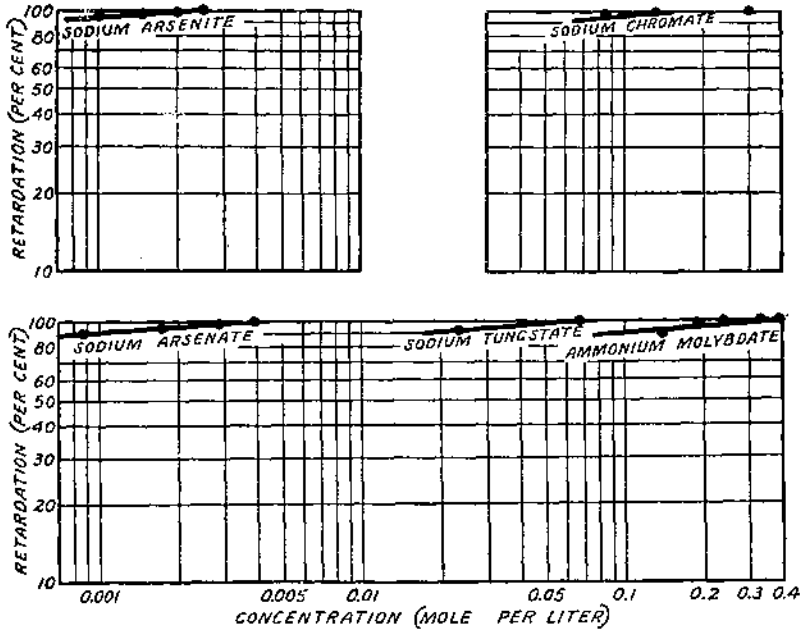


FIGURE 15.—Concentration-retardation curves for inorganic materials with poisonous acid radicals against the fungus *Fomes annosus*

TABLE 7.—*The most probable total inhibition points of para substituted benzene derivatives*

Compound	Highest concentration at which fungus grew	Lowest concentration at which no growth was obtained	Most probable total-inhibition point	Compound	Highest concentration at which fungus grew	Lowest concentration at which no growth was obtained	Most probable total-inhibition point
	Mole per liter	*Mole per liter*	*Mole per liter*		*Mole per liter*	*Mole per liter*	*Mole per liter*
p-chlorophenol	0.00125	0.00145	0.0013	*p*-chloroaniline	0.0032	0.0039	0.0039
p-nitrophenol	----------	.00107	.0009	*p*-nitroaniline	.0044	.0046	.0046
p-aminophenol	.0275	.036	.031	Hydroquinone	.036	.045	.039

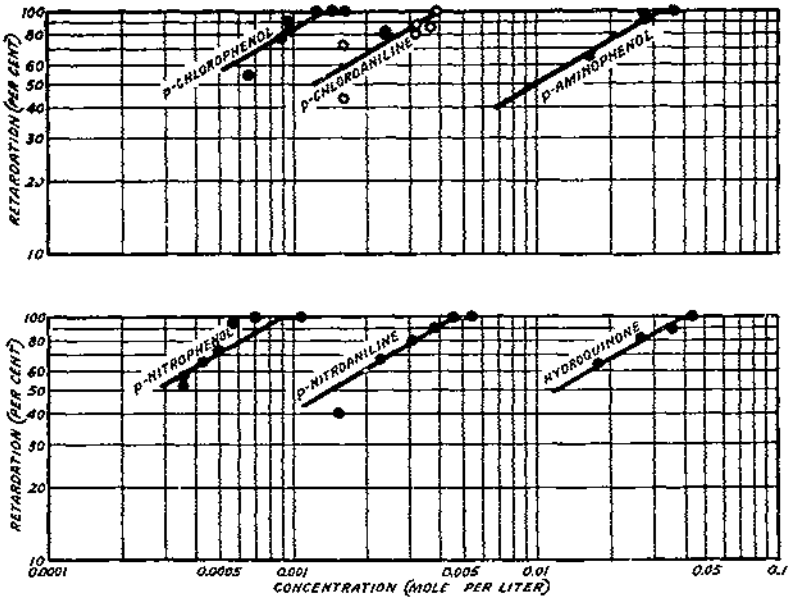


FIGURE 16.—Concentration-retardation curves for para substituted benzene derivatives against the fungus *Fomes annosus*

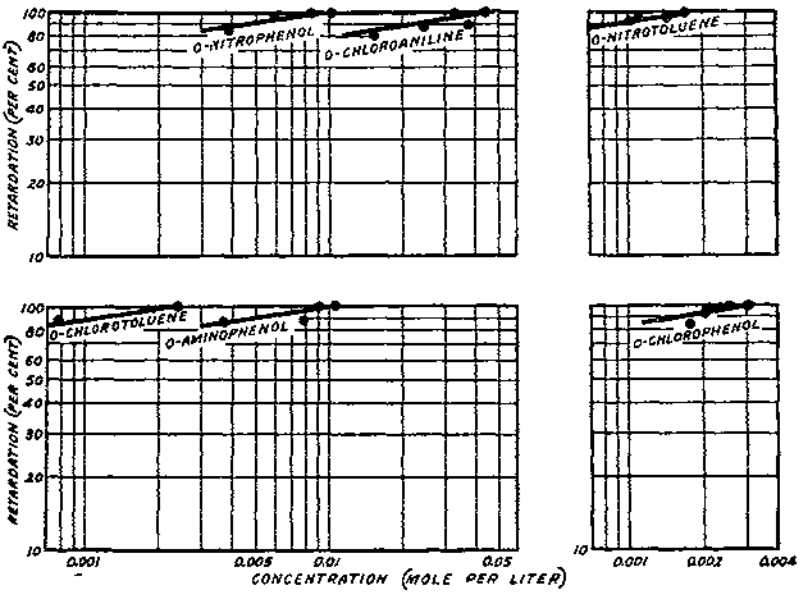


FIGURE 17.—Concentration-retardation curves for ortho substituted benzene derivatives against the fungus *Fomes annosus*

TABLE 8.—*The most probable total inhibition points of ortho substituted benzene derivatives*

Compound	Highest concentration at which fungus grew	Lowest concentration at which no growth was obtained	Most probable total-inhibition point	Compound	Highest concentration at which fungus grew	Lowest concentration at which no growth was obtained	Most probable total-inhibition point
	Mole per liter	*Mole per liter*	*Mole per liter*		*Mole per liter*	*Mole per liter*	*Mole per liter*
o-chlorotoluene	0.00078	0.0024	0.0024	o-nitrotoluene	0.00145	0.0017	0.0017
o-nitrophenol	.00084	.0014	.0009	o-aminophenol	.0092	.0108	.0105
o-chlorophenol	.0026	.0031	.0031	o-chloroaniline	.0038	.0044	.0044

There was one major exception to the behavior of ortho and para substituted compounds. This occurred in the salts of the substituted benzoic acids. Before these can be discussed it is necessary to present the data for the monosubstituted derivatives of benzene shown in Figure 18.

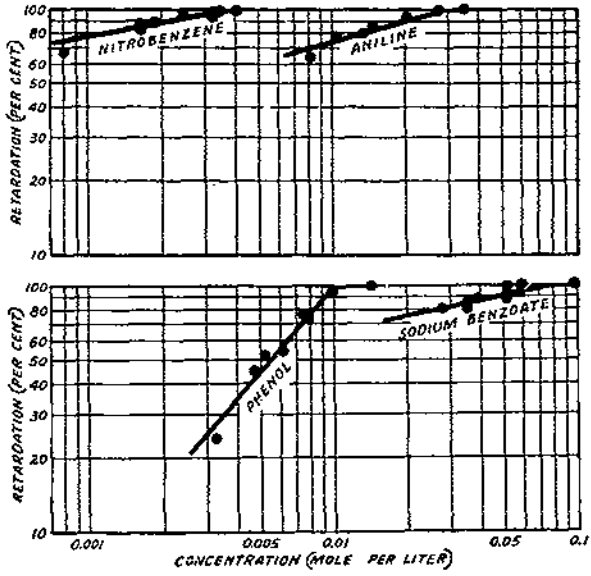


FIGURE 18.—Concentration-retardation curves for monosubstituted benzene derivatives against the fungus *Fomes annosus*

The total-inhibition points are given in Table 9. In the monosubstituted compounds there seems to be no general slope that will fit all the data; the structure of the four monosubstituted derivatives may be represented as follows:

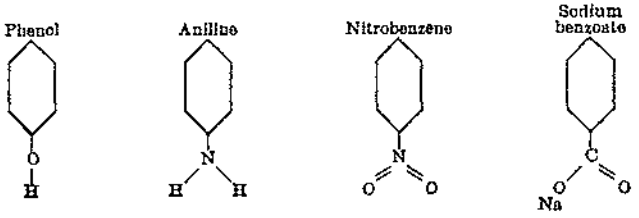


TABLE 9.—*The most probable total-inhibition points for monosubstituted benzene derivatives*

Compound	Highest concentration at which fungus grew	Lowest concentration at which no growth was obtained	Most probable total-inhibition point	Compound	Highest concentration at which fungus grew	Lowest concentration at which no growth was obtained	Most probable total-inhibition point
	Mole per liter	*Mole per liter*	*Mole per liter*		*Mole per liter*	*Mole per liter*	*Mole per liter*
Sodium benzoate	0.06	0.098	0.08	Phenol	0.01	0.0145	0.0102
Aniline	.028	.035	.03	Nitrobenzene	.0041	(1)	.0042

[1] No normal growth was obtained at 0.0044 and 0.0054 mole per liter, but abnormal spore growth was obtained. At 0.0065 mole per liter no growth of any kind was obtained, and the fungus was dead.

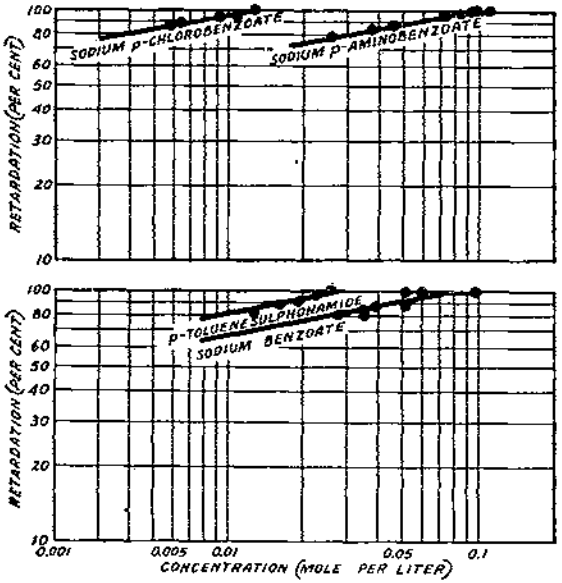


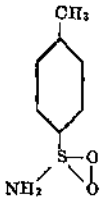
FIGURE 19.—Concentration-retardation curves for salts of benzoic and substituted benzoic acids

In Figures 16 and 17 it was shown that when combinations of any two of amino, chlorine, hydroxy, nitro, or methyl groups were present in the same molecule the slope of the line representing the effect of concentration on the percentage retardation seemed to be dependent upon the relative positions of the two groups. This was not so with the sodium salts of substituted benzoic acids, the slopes of which were more nearly parallel to the slope of sodium benzoate as shown by Figure 19. The total-inhibition points of these compounds, determined graphically, are given in Table 10.

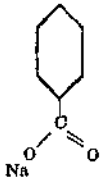
TABLE 10.—*The most probable total inhibition points of the salts of benzoic and substituted benzoic acids*

Compound	Highest concentration at which fungus grew	Lowest concentration at which no growth was obtained	Most probable total-inhibition points	Compound	Highest concentration at which fungus grew	Lowest concentration at which no growth was obtained	Most probable total-inhibition points
	Mole per liter	*Mole per liter*	*Mole per liter*		*Mole per liter*	*Mole per liter*	*Mole per liter*
Sodium benzoate	0.06	0.098	0.082	Sodium *p*-chlorobenzoate	0.011	0.0128	0.0128
Sodium *p*-aminobenzoate	.1	.11	.11	*p*-toluenesulphamide	.026	.....	.030
Sodium *m*-aminobenzoate	.084	.094	.094				

Another exception is *p*-toluenesulphonamide. This may be looked upon as the ammonium salt of *p*-toluenesulphonic acid from which one molecule of water has been lost. Its structure may be represented by:



which is very similar to that of sodium benzoate:



P-toluenesulphonamide has a physiological action very similar to that of sodium benzoate, suggesting that all the sulphonic acid salts may behave in a very similar manner.

DISCUSSION OF EXPERIMENTAL RESULTS

The departures of several of the points in Figures 14 to 19 from the lines averaging the series to which the points respectively belong are somewhat greater than can be attributed to experimental error. Most of these points occur at the lower concentrations. This seems to indicate that in such materials the effect of low concentrations is quite different from that of high concentrations in the rate at which the toxic effect increases with concentration.

Whatever may be the cause of this apparent change in behavior, it is sufficient here to limit the discussion to the region over which the parabolic relationship shown by the experimental work holds good. At present these limits appear to be as follows: Between 60 and 100

per cent retardation for para compounds, or when the slope of the toxicity concentration curve is 0.56; between 80 and 100 per cent retardation for ortho compounds, or when the slope is 0.15; between 80 and 100 per cent retardation for substituted benzoic acids, or when the slope is 0.19; between 20 and 100 per cent retardation for inorganic basic radicals, or when the slope is 1; and between 90 and 100 per cent retardation for inorganic acid radicals, or when the slope is 0.07. When stated in terms of concentration, the relationship seems to hold in all compounds between the concentrations producing total inhibition and a concentration only one-third as great.

The straight lines shown in the logarithmic plots, such as in Figures 14 to 19, are equivalent to parabolic or simple-power curves on ordinary cross-section paper. The equation of such a curve may be written $R=DC^n$ in which R is the percentage retardation and C is the concentration. The exponent n is the slope of the line on logarithmic paper, and D is a constant or parameter whose value depends on the position of this line. The experimental work indicates that one value of n is applicable to a group of chemicals of similar constitution. It may, therefore, be considered that n is determined by the structure, or configuration, and D by the composition of the chemical.

CONCLUSIONS FROM EXPERIMENTAL WORK

The following conclusions can be drawn from the experimental work on *Fomes annosus:*

The radial growth of the fungus *Fomes annosus* is directly proportional to the time of growth, provided other conditions are the same.

There is a definite relationship between the concentration and the percentage retardation of the growth of *Fomes annosus* grown on nutrient agar containing various chemicals.

This relationship may be expressed as a straight line on logarithmic paper and holds true apparently between the total inhibition point and approximately one-third of that concentration. For this portion a parabolic relation apparently exists.

It is possible to arrange chemicals in groups whose percentage retardation-concentration curves are similar in slope and in which the chemical constitution is also similar.

The relationship between concentration and percentage retardation may be expressed by the general equation

$$R=DC^n$$

in which R is the percentage retardation, C the concentration, n a constant or exponent which seems to be a function of the chemical structure, and D a constant which seems to be dependent upon the individual compound.

WORK OF OTHER INVESTIGATORS

A comprehensive search of all the literature to find whether similar relationships exist in other organisms is well-nigh impossible and has not been attempted. In fact, only a small portion of the literature has been reviewed and even a smaller portion of that reviewed has been used. It is believed, however, that the data presented in this

critical review are sufficient to show the possibility of a wider application of the scheme of analysis used here and to aid in the development or extension of relationships which were beyond the scope of the original data. In dealing with different organisms, different methods of measuring toxicity must be used. In general, the toxicity is measured by whatever convenient measure is available. If the toxicity data are of such a nature that they can be put into terms comparable with the percentage retardation of the previous discussion, it is possible to determine whether a similar relationship exists between toxic effect and concentration. Arranging chemicals in groups of like constitution and like rate of change in toxic action with changes in concentration is a more difficult task, because it requires a sufficient number of chemicals of like constitution to establish the best relationship, and also because there is no reason to believe that the relationship shown in the experimental work on fungi would hold in exactly the same manner with other organisms. In fact, it would be surprising if this were true, but some other arrangement of like chemicals in groups showing, in general, the same sort of influence, though with different numerical value, would not be surprising. But, as stated previously, a sufficient number of tests would be required to establish the relationship. Such data are not likely to be found in the literature unless some other investigator has proceeded along a similar line of attack as that followed in this bulletin.

In attempting to find the relationship in toxicity data with other organisms, cognizance must be taken of the fact that in some toxicity work the organisms normally live beyond the length of the experiment, while in other work the organisms normally die under the conditions of the test, even if no toxic agent is present.

APPLICATION OF THE ANALYSIS TO DATA ON BEAN APHIS

Tattersfield, Gimingham, and their coworkers have conducted extensive toxicity tests (*15*, *16*, *17*) using insects and worms as their organisms. Since such organisms are representatives of the animal kingdom whereas the wood-destroying fungus *Fomes annosus* represents the vegetable kingdom, the data are of particular interest because any scheme of analysis that can be used on the toxicity data from two such widely separated groups must approach the fundamentals of toxicity as a whole. Many of the data collected on the bean aphis (*Aphis rumicis*) by these workers are not susceptible to the mathematical treatment proposed here because, for the most part, they record only the lethal concentration and give no idea of the relative effect of concentrations below lethal doses. Tattersfield, Gimingham, and Morris report experiments (*16*, *p. 220*) on more than 60 organic compounds with approximately 10 aphids for each compound. The accidental death of two aphids would, of course, introduce a large error in their data. This is perhaps the reason why the authors state:

> It should be pointed out that the curves on the diagrams are not suitable for mathematical analysis and can not be used for exact interpolation; they are intended to show the general trend of the experimentally determined points * * *.

The relationship between toxic action and concentration under the scheme of analysis used here does not require the exact determination of the killing point, but does require that a sufficient number of deter-

minations be made below the killing point so that the trend of the effect of the concentration can be determined.

As much of Tattersfield's data as possible has been taken, keeping in mind that the data can not be more accurate than 10 per cent and that a 20 per cent error would probably be within the limit of the occasional experimental error. Tattersfield's data give both the number of survivors and the number dead or dying. The number dead when expressed on a percentage basis is similar to the percentage retardation, if it can be assumed that the weak aphids die at lower concentrations than the stronger ones. The method of plotting also

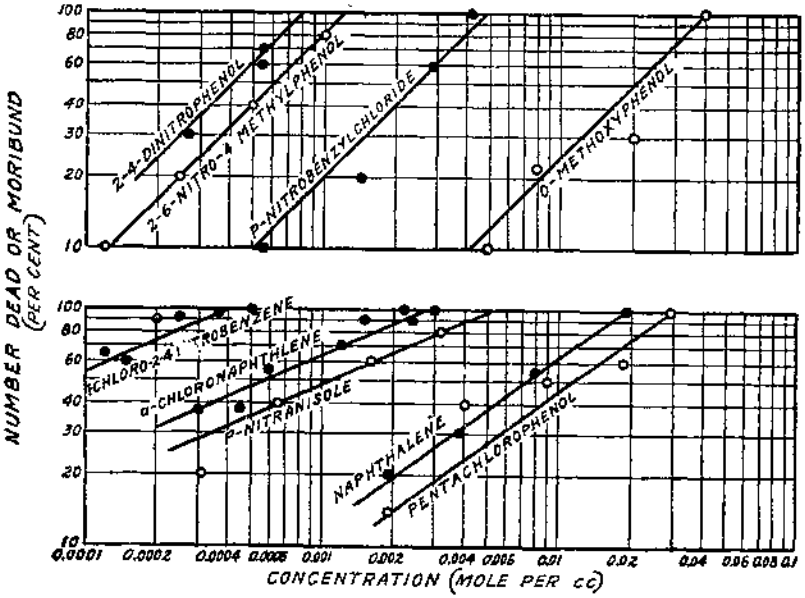


FIGURE 20.—Concentration-death curves for various chemicals against the bean aphis (*Aphis rumicis*). From data by Tattersfield, Gimingham, and Morris (*16*)

requires that at least three determinations be recorded below the concentration that was lethal to all the subjects investigated. This condition is fulfilled by only 13 of the pure organic materials used by Tattersfield. The curves for 9 of the 13 materials are given in Figure 20. The others have been omitted for the sake of clearness of the figures. Only four of the points shown in Figure 20 are farther away from the lines drawn than would be accounted for by the death of one aphid. Two of the four points in the curve are for *o*-methoxyphenol. The actual record taken from the original paper and the number of deaths required by the curve for this chemical are shown in Table 11. The best agreement is furnished by 2-6 nitro- 4 methylphenol, which gives a straight line including all four points.

TABLE 11.—*Comparison between the number of deaths obtained by experiment* [1] *and that required by the curve for o-methoxyphenol*

Concentration	Number unaffected	Number dead	Required by curve	Difference
Mole per 100 c c				
0.0403	0	10	10.0	0.0
.0201	7	3	4.7	1.7
.008	7	2	1.7	.3
.004	9	1	1.0	.0
[2].002	8	2	.5	1.5

[1] From data by Tattersfield, Gimingham, and Morris (*16*).
[2] This point not plotted in Figure 20.

The data furnished by Tattersfield and his coworkers on the toxicity of the alkaloids (*16*) also plot as straight lines on logarithmic paper. (Fig. 21.) Their data on the toxic effect of various plant

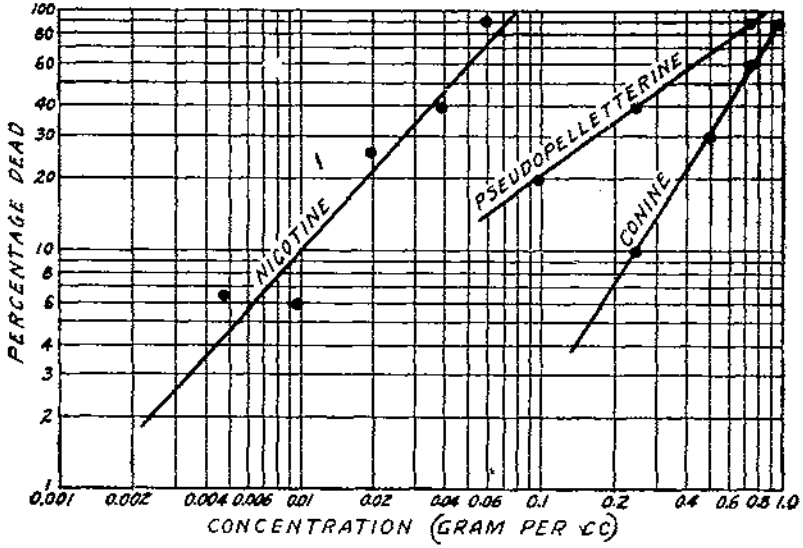


FIGURE 21.—Concentration-death curves of alkaloids against the bean aphis (*Aphis rumicis*). From data by Tattersfield, Gimingham, and Morris (*16*)

extracts, and the like, need not be considered here because the extracts were necessarily impure toxic substances. These are outside of the scope of the present discussion since their consideration necessarily leads to a discussion of the complex relationship that must exist when two or more dissimilar toxic materials are mixed together or when a water-soluble toxic material is dissolved in an oil.

Of the 9 curves shown in Figure 20, 1 is the hydrocarbon naphthalene, 1 is an alpha substituted naphthalene, 1 is an ortho substituted benzene, 2 are para substituted benzenes, 2 are trisubstituted benzenes, 1 a tetrasubstituted benzene, and 1 a hexasubstituted benzene. In view of the meagerness of the data it is not possible to arrange the compounds into groups of like or nearly like constitution. It is believed, however, that there is sufficient evidence to show that the parabolic relationship between changes in concentration and toxic effect holds here as with fungi.

APPLICATION OF THE ANALYSIS TO DATA ON THE BACTERIA STAPHYLOCOCCUS PYOGENES AUREUS AND BACILLUS PARATYPHOSUS AND ON ANTHRAX SPORES

The conditions of many toxicity tests in which bacteria are used as test organisms are such that the bacteria would slowly die without the aid of the toxic material. The effect of the toxic material is therefore to increase the death rate. It is essential to know something about the death rate under the conditions of the tests before any attempt is made to determine whether or not the parabolic relationship between concentration and toxic effect holds with bacteria also.

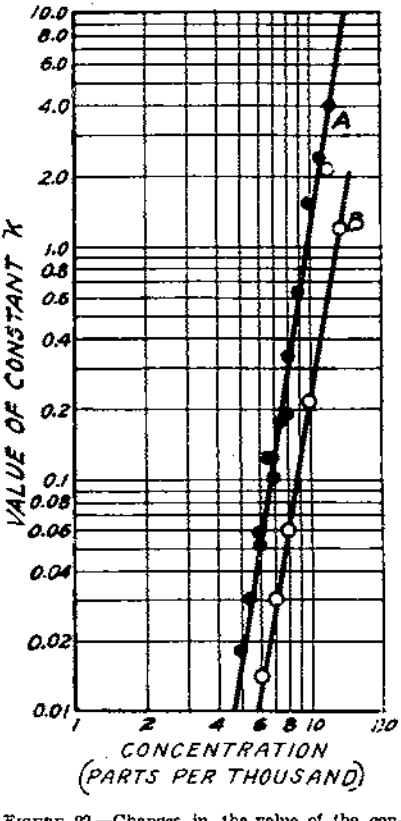


FIGURE 22.—Changes in the value of the constant K with changes in concentration of the disinfectant (phenol). A, against the bacterium, *Bacillus paratyphosus;* B, against the bacterium, *Staphylococcus pyogenes aureus.* From data by Chick (*1*). The data for A are calculated to the logarithm base e, while those for B are calculated to the base 10. This avoids interference of one curve with the other. The data for B can be converted to the base e by multiplying by 2.30

Chick (*1*), following the suggestion given by Krönig and Paul (*9*), found that the viability curve of bacteria can, in general, be represented by the equation of a monomolecular reaction,

$$a-x=ae^{-Kt}$$

or

$$K=\frac{1}{t}\log\frac{a}{a-x}$$

Where a is the number of organisms at the beginning; x the number dead at the end of any time, t; and K a constant which expresses the death rate for the particular condition under which the test was run. This representation of the viability curve of bacteria by the monomolecular equation was later substantiated by Cohen (*2*) and by Winslow and Falk (*18*), although the latter paper notes numerous apparent exceptions.

Any change in the conditions of the test changes the value of the constant K since it changes the rate of death. The constant K of those conditions must, therefore, contain the factor K_1 for the normal death rate and P for the percentage effect of the poison. This part of the equation could therefore be written

$$K=K_1\ (1.00+P)$$

or

$$K=K_1+K_1P$$

If K_1 were known, it would be very simple to calculate the values of P and the experimental value of K, but Chick does not give any

value from which K_1 could be calculated. If, however, the value of K_1 is small in comparison to the values of K_1P it can be neglected and therefore K can be taken as equal to K_1P. Since P is comparable to percentage retardation, the value of K should plot as a straight line on logarithmic paper, because the multiplication of P by any other constant will not change the slope of the parabola that might be formed by the various values of P obtained from different concentrations.

Chick, working with *Bacillus paratyphosus* and *Staphylococcus pyogenes aureus* tested the toxic action of phenol against these organisms. She presented evidence to show that the monomolecular-reaction equation could be modified to fit the various conditions of her experiments. It is not the purpose here to question the validity of Chick's modified equation, but merely to point out that the relationship shown in the experimental part of this bulletin also fits the data. To this end the values of the constant K for each concentration of phenol have been worked from Chick's original data as given in Tables 12 and 13, and plotted against their concentration, the assumption being that K_1 was small. Part of these data are shown in Figure 22. It is evident from the steepness of the line in Figure 22 that K_1 must be small because K equals K_1+K_1P has a value of only 0.01 at a concentration of 4, and at a concentration of 2 parts per thousand would have a value of 0.001. They show a parabolic relationship between the concentration and the value of the constant as is required by the relationship.

TABLE 12.—*Time required for killing 30,000,000 Staphylococcus pyogenes aureus at 20° C. with varying concentrations of phenol* [1]

Phenol parts per 1,000 (C)	Time taken for disinfection (t)		Phenol parts per 1,000 (C)	Time taken for disinfection (t)	
	Hours	*Minutes*		*Hours*	*Minutes*
14.0	0	4.5	7.0	3	0.0
12.5	0	2.5	6.0	6	35.0
10.0	0	25.0	[2] 4.0	23	42.0
8.0	1	35.0			

[1] From data by Chick (*1*).
[2] This point not plotted. It is slightly above the curve.

TABLE 13.—*Time required for reduction in number of about 30,000,000 Bacillus paratyphosus at 20° C. to less than 60 with varying concentrations of phenol* [1]

Phenol parts per 1,000 (C)	Time taken for disinfection (t)		Phenol parts per 1,000 (C)	Time taken for disinfection (t)	
	Hours	*Minutes*		*Hours*	*Minutes*
12.0	0	3.25	[2] 3.0	75	0
11.0	0	5.5	8.0	0	45.0
10.0	0	8.5	7.5	1	15.0
9.0	0	20.5	7.0	1	45.0
8.0	1	8.0	6.5	2	5.0
7.0	2	0.0	6.0	3	45.0
6.0	4	0	5.5	7	20.0
[2] 4.5	19	0	5.0	11	30.0
[2] 4.0	19	0			

[1] From data by Chick (*1*).
[2] These points not plotted. The point for 4.5 parts of phenol is on the line; the other 2 are slightly above.

Chick also reproduces the data of Krönig and Paul on the effect of mercuric chloride on anthrax spores. These data have been handled in a manner similar to that described for Chick's data. The values of the constants K for Krönig and Paul's data are plotted against their concentrations in Figure 23.

From these data it appears more than likely that the parabolic relationship shown in the experimental work on fungi can be used with satisfactory results when bacteria are the test organisms. The complete equation for the viability of bacteria may therefore be written as

$$DC^n = \frac{1}{t} \log \frac{a}{a-x}$$

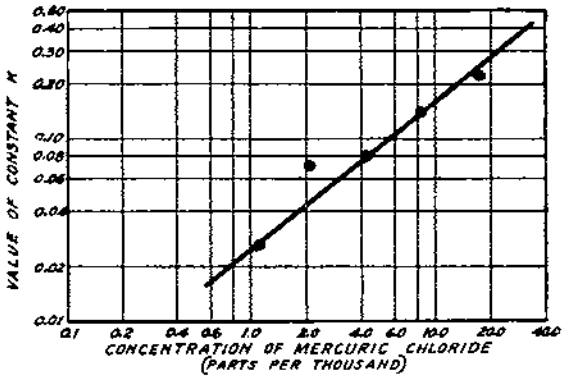


FIGURE 23.—Changes in the value of the constant K with changes in concentration of mercuric chloride against anthrax spores. From data by Krönig and Paul (9)

where DC^n is equal to P if the normal viability constant is small, and as

$$K_1 (1.00 + DC^n) = \frac{1}{t} \log \frac{a}{a-x}$$

if the normal viability constant K_1 is large in respect to DC^n.

This last form is similar to the equation of growth for fungi as given on page 18.

$$K_m (1.00 - DC^n) = \frac{1}{t} G$$

The left side of the equation, which is the side governing the rate, differs only in the sign. The viability curve for the fungus was a life curve and was opposed by the toxic action; the viability curve of the bacteria was a death curve and was aided by the toxic action.

APPLICATION OF THE ANALYSIS TO DATA ON GREEN PLANTS

The effect of different materials upon growing plants has long been of interest to mankind because the whole science of fertilizers is bound up with the effects of lime, magnesia, ammonia, and the like, upon growing plants. These effects are of particular interest to those plants commonly used as foods. McCool (12) measured the effect of various poisons on peas and wheat, grown in distilled water,

Pfeffer's nutrient solution composed entirely of inorganic salts in distilled water, and in soil. He measured the weight of the green tops, the weight of the green roots, and the average length of both roots and tops. Because of the wide range of the investigations and because in many cases mixtures of two bases were used, not all of his data were valuable for use here.

Figure 24, which has been plotted from McCool's data, shows the effect of increasing the concentration of manganese chloride on the weight of the tops of peas grown in nutrient solution. The data are given in Table 14.

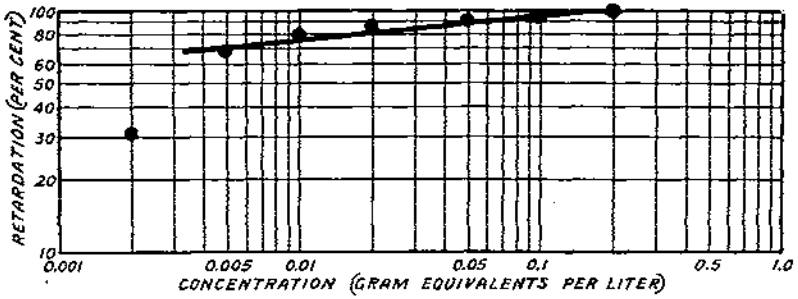


FIGURE 24.—Concentration-retardation curves for manganese chloride against Canada field peas grown in nutrient solution. From data by McCool (*12*)

TABLE 14.—*Effect of manganese chloride on the weight of tops of peas*

Concentration of manganese chloride	Weight of green tops [1]	Retardation		Concentration of manganese chloride	Weight of green tops [1]	Retardation	
N	*Grams*	*Grams*	*Per cent*	*N*	*Grams*	*Grams*	*Per cent*
[2]0	10.40	0	0	0.020	1.50	8.90	85.6
.002	7.20	3.20	30.8	.050	.85	9.55	91.8
.005	3.20	7.20	69.2	.100	.64	9.76	94.0
.010	2.20	8.20	78.8	.200	.00	10.40	100.0

[1] From data by McCool (*12*). [2] Nutrient solution.

The parabolic relationship given on page 18 expresses the data from the total inhibition point to approximately one-fifteenth of that concentration as accurately as the experimental data will allow. The effect of changing the concentration of the posion shown here has also been confirmed in other examples on green plants. The toxic effect can therefore be considered as a parabolic function of the concentration of the poison for a very considerable part of the range.

EXTENSION OF THE ANALYSIS TO STIMULATION

A discussion on the general effect of changes in the concentration of a toxic material can not well be concluded without some consideration of the stimulating effect of low concentrations. This is particularly true here, where it has been shown that an increase in concentration causes an increase in toxic effect and that within certain limits the increase in toxic effect appears to be a simple parabolic function of the concentration. Since a parabolic function does not

permit of any reversal in the curve, no stimulation can be accounted for by this simple parabolic function. However, the fact that poisonous materials when used in low concentrations frequently act as stimulants is well known. Winslow and Falk (*18*) ascribe to Richet the discovery that there are certain concentrations of the same salts which are (1) indifferent, (2) stimulating, (3) inhibitive, and (4) toxic. Winslow and Falk point out that the change from a stimulating to a retarding influence is apparently a continuous process and that the influence can be represented by smooth curves. It is extremely difficult for the chemist to visualize a single reaction that proceeds in one direction at one concentration and in the entirely opposite direction at some other concentration. There are a few cases in colloid chemistry where apparently one concentration of a foreign material peptizes the colloid and another concentration coagulates it.

The conception of two opposed reactions can easily be applied to the effect of changes in concentration on the welfare of living organisms if it is assumed that both reactions are present at all concentrations of the reacting material. If one of these reactions is beneficial and the other detrimental to the well-being of the organism, the one which has the greatest effect at any particular concentration will be the one whose influence is more apparent at that particular concentration. If the two reactions are equal at some concentration, there will be no apparent effect. This bulletin has shown that the detrimental effect of a poison on a fungus, on aphids, on bacteria, and on green plants is very closely approximated by a parabolic function of the concentration, at least within the range of the data shown.

If this range is extended into the region where stimulation is obtained the whole range of change in concentration can be represented by two parabolas, one representing a detrimental and the other a beneficial effect. At no concentration can either effect be measured alone, because of the presence of the other opposing effect. The measurable results are the net resultant of these two opposed reactions. When the beneficial reaction is greater than the detrimental reaction the net result will be stimulation. When the detrimental reaction is greater than the beneficial reaction the net result will be a toxic action. At the point at which the two are equal there will be no measurable effect. The net stimulation must be added to the figure representing the growth of the organism in the absence of the chemical under investigation, and the net detrimental effect must be subtracted from this figure. When the net detrimental effect equals the normal growth, no growth or total inhibition is obtained. The measurable detrimental effect can not therefore exceed the normal growth, but the net stimulation may exceed the normal growth. No such limitations apply to either effect if taken by itself; the detrimental effect, if taken by itself, may be many times the normal growth provided it is opposed by a beneficial effect which is great enough to bring the difference between the two effects within the range of measurement. Since the beneficial and detrimental reactions have different rates, the curves representing their plotting on logarithmic paper must cross.

Some explanation is necessary, since a hypothesis is now offered which proposes to represent by the difference between two crossed parabolas the same effect that was previously represented by a single

parabola. Fortunately, this conception of two crossed parabolas is the explanation of the exceptions and discrepancies noted in the early part of the bulletin. Figure 25 shows three different pairs of crossed parabolas representing beneficial and detrimental reactions. The difference between the two is the net beneficial and net detrimental effect. In these figures the net beneficial effect, or stimulation, is always on the left side of the crossing point of the two lines. This is added to the normal growth. The net detrimental effect is always on the right side of the crossing point. It must be subtracted from

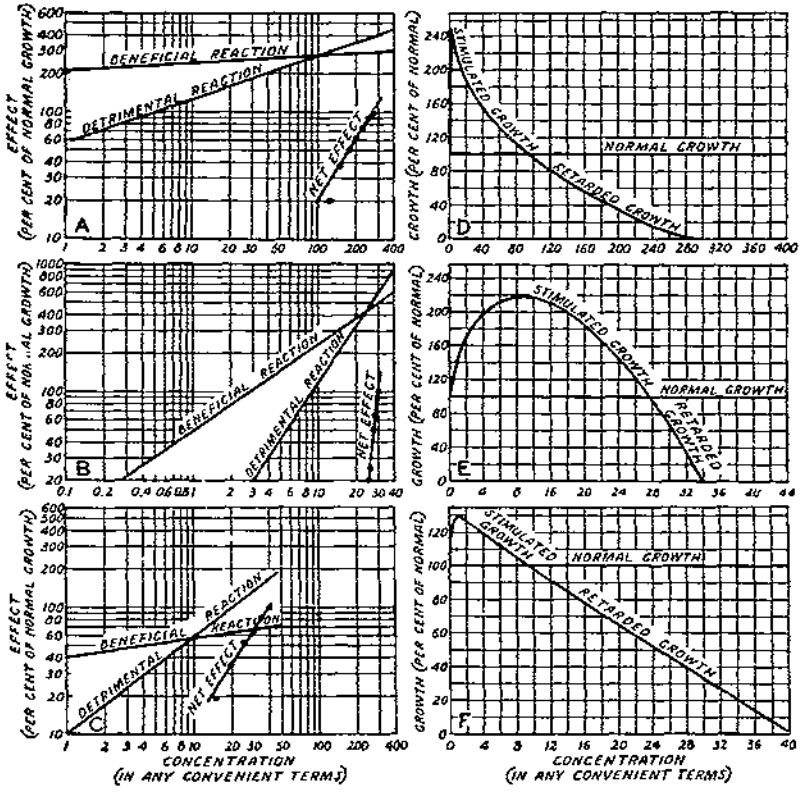


FIGURE 25.—Illustration of the hypothesis of beneficial and detrimental effects used as parabolic functions of the concentration: A, B, and C, crossed parabolas; D, E, and F, the corresponding growth curves. The heavy line with dots represents the difference between the two parabolas when the net effect is detrimental

the normal growth. The net detrimental effect obtained by these pairs of crossed parabolas is shown by the heavy line with dots in Figure 25. Any investigator collecting data within the range of toxic effect would be justified in drawing a straight line through the experimentally determined points represented by the dots because the differences of the experimental data are less than 5 per cent from the line, and are within the limit of error of most experimental toxicity data. A close examination of the points in Figure 25 shows that the data are not true straight lines but are curves and that the points have a tendency to be above the line in the center and below the line at both ends. In experimentally determined points the experimental

error may throw the point on either side of the line, and a straight line relation would be even more justified.

In order that the idea of crossed parabolas may be more clearly depicted the curves in Figure 25, A, B, and C, have been replotted on ordinary coordinate paper as total growth lines. (Fig. 25, D, E, and F.) The normal growth is represented by a horizontal line drawn at 100 per cent. The portion above this line is the difference between the two parabolas at the left side of the crossing point added to 100 per cent. The portion below this line is the difference between the two parabolas at the right side of the crossing point subtracted from 100 per cent.

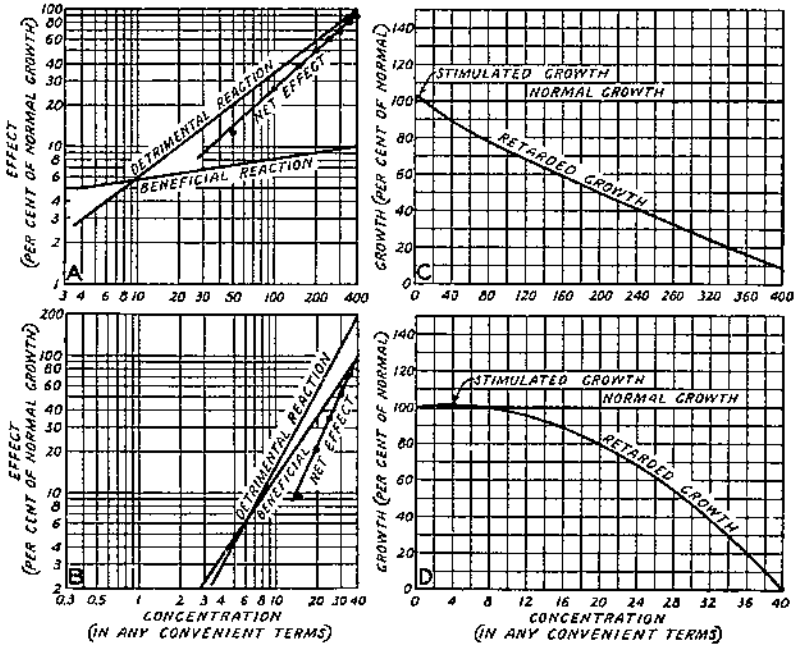


FIGURE 26.—Illustration of the hypothesis of beneficial and detrimental effects used as parabolic functions of the concentration when there is no measurable stimulation: A and B, crossed parabolas; C and D, the corresponding growth curves. The heavy line with dots represents the difference between the two parabolas when the net effect is detrimental

One objection to the hypothesis of crossed parabolas is that some organisms do not show a net stimulation with certain chemicals; others may not show a stimulation with any materials even when the whole range is carefully investigated. In Figure 26, curves A and B show two pairs of crossed parabolas representing opposed beneficial and detrimental effects. The growth curves for the crossed parabolas in Figure 26 are shown opposite them in C and D. Both pairs of curves show a stimulation which is less than the experimental error and would ordinarily be missed or considered as erroneous. In Figure 26, D, this condition exists for over one-fourth the distance between zero concentration and the total inhibition point. The objection that no stimulation can be shown, therefore, does not eliminate the possibility that the net effect can be depicted by crossed parabolas. Curve C of Figure 25 showing stimulation and curve A of

Figure 26 showing no measurable stimulation are made up of two pairs of crossed parabolas having the same slopes, but different intercepts. The point of crossing in curve C is at concentration 9.5 and percentage effect 56; in curve A the parabolas cross at concentration 9.5 and percentage effect 5.6.

APPLICATION OF THE HYPOTHESIS TO BACTERIUM COLI

The data presented by Hotchkiss (*6*) afford an excellent opportunity of testing out the hypothesis of two reactions with *Bacterium coli*. Hotchkiss measured the effect of a number of cations on the number of bacteria present at stated intervals. For the most part her data show only the numbers present at one time during the test. The time of the test is, therefore, of no interest in this connection, provided that the same interval is used for each concentration. For convenience, her data have been recalculated into percentage of the normal numbers alive as indicated by the control cultures that were run at the same time. When the percentage alive exceeds 100 per cent of the normal, the stimulating effect was greater than the detrimental effect and, conversely, when the percentage alive was less than 100, the detrimental effect was the greater.

There is at present no simple way of calculating the real values of the beneficial and detrimental reactions from data as collected. It is, however, very simple to pick out by "cut and try" methods a pair of parabolas that will fit the data for each compound.

Some of the data collected by Hotchkiss (*6*) have been so plotted and the results of this method of "calculation" are shown in Table 15 along with the original data recalculated to percentage effect.

TABLE 15.—*Comparison between the calculated and experimental values of the effect of various concentrations of inorganic salts on the viability of Bacterium coli*

Salts	Concentration	Number surviving after 6 hours		Salts	Concentration	Number surviving after 6 hours	
		Calculated	Observed[1]			Calculated	Observed[1]
	Mole per liter	*Per cent*	*Per cent*		*Mole per liter*	*Per cent*	*Per cent*
Magnesium chloride	0.0125	148	147	Zinc chloride	0.000001	146	139
	.025	160	160		.000005	136	130
	.050	172	190		.00001	124	73
	.105	173	175		.00005	52	57
	.25	95	96		.0001	0	0
	.50	0	0	Stannic chloride	.000005	141	142
Nickel chloride	.000005	170	175		.00001	140	142
	.00001	175	175		.00005	134	142
	.00005	181	175		.0001	127	100
	.0001	172	175		.0005	80	83
	.0005	110	112		.0011	40	58
	.001	50	50		.005	0	0
	.005	0	0				

[1] From data by Hotchkiss (*6*).

APPLICATION OF THE HYPOTHESIS TO THE SPROUTING OF WHEAT

The work of Jensen (*8*) furnishes an example of the application of the crossed parabolic hypothesis to green plants. Jensen used sprouted wheat seedlings in water solutions, in nutrient solutions, in sand, and in soil. He varied the concentration of the salt and measured the average length of sprout, the total transpiration, and the weight of the test specimens when green and also when dry. The grain was

sprouted before the experiments started, but since there are no data on the average dry weight or green weight at the beginning of the experiment, these figures can not be used. The statement is made, however, that the sprouts were approximately 3 mm long when the experiment was begun. The growth which takes place during the experiment is the total length recorded minus the length at the beginning. In this case, therefore, the length factor is the only factor which can be used to determine the effect of the chemical on the growth of the organism, and even this is only approximate because it must be assumed that all of the sprouts were 3 mm long. The process of preparing the data for use in this mathematical treatment involved the following steps: (1) A determination of the increase of length that took place during the experiment (total length minus 3 mm). (2) A determination of the percentage increase or decrease over the normal growth expected. (3) The empirical fitting of the data by two crossed parabolas. (4) Reading from these theoretical curves the percentage increase or decrease in the growth due to the presence of different concentrations of poisons. (5) Reconverting the percentage effect into length measurements and adding to this figure the 3 mm which was subtracted at the beginning of the calculation. This procedure gives the total length of the wheat sprout required under the theory of two reactions, one of which is stimulating, the other detrimental, and also shows that both reactions are parabolic functions of the concentration. A comparison of the results obtained by this process of calculation and empirical fitting of the data when the wheat sprouts were grown on a lead nitrate solution of various concentrations is given in Table 16. A similar comparison between the theoretical requirement and the experimental result when the wheat was grown on a nutrient solution containing various concentrations of zinc sulphate is given in Table 17.

TABLE 16.—*Comparison of calculated and experimental determination of length of wheat sprouts grown in lead nitrate solution*

Concentration	Length calculated	Length observed [1]	Concentration	Length calculated	Length observed [1]
N	*Cm*	*Cm*	*N*	*Cm*	*Cm*
0.0001	12.43	12.47	0.006	8.53	8.15
.0002	12.22	11.35	.008	9.00	8.9
.001	11.55	10.2	.01	8.60	8.02
.002	10.05	10.4	.02	7.1	7.3
.004	10.05	9.75	.04	5.3	5.3

[1] From data by Jensen (*8*).

TABLE 17.—*Comparison of calculated and experimental values of the length of wheat sprouts grown in zinc sulphate in a nutrient solution*

Concentration	Length calculated	Length observed [1]	Concentration	Length calculated	Length observed [1]
N	*Cm*	*Cm*	*N*	*Cm*	*Cm*
0.00001	13.85	13.85	0.0009	11.16	11.47
.00002	13.81	13.30	.001	10.95	10.57
.00005	13.63	14.70	.002	9.01	9.10
.0001	13.42	15.25	.004	6.70	6.90
.0003	12.69	12.77	.005	5.88	5.97
.0005	12.08	12.20	.006	4.94	5.02
.0007	11.98	11.45	.007	4.10	4.57

[1] From data by Jensen (*8*).

When, however, the plants are grown on soil the data do not follow the same laws. Jensen (*8*), however, shows that even with pure sand there is a considerable surface adsorption, so that the concentrations he records as being used in soil were certainly not the concentrations that were available to the plants. If there were a means of calculating the surface adsorption, a closer agreement might be obtained with soils. These results, while they do not prove the existence of two opposed reactions, at least show that the hypothesis is tenable and warrants further consideration.

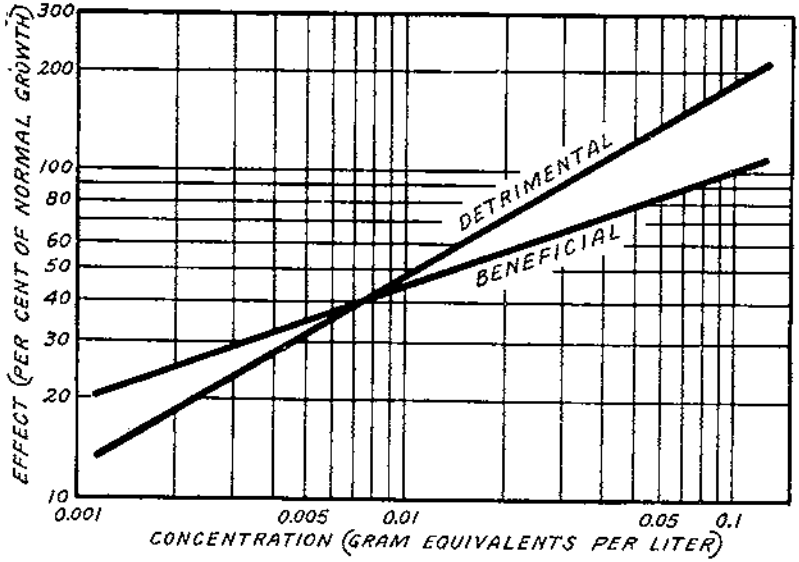


FIGURE 27.—Curves representing a beneficial and detrimental effect of manganese chloride on Canada field peas grown in soil. From data by McCool (*12*)

APPLICATION OF THE HYPOTHESIS TO GROWING PEAS

The discussion of the work of McCool (p. 33) was based on the assumption of a parabolic relationship between concentration and toxic effect. The chemicals previously discussed, however, showed the existence of a stimulating effect when used by McCool under different conditions of the experiment. In Figure 24 McCool's results on growing peas in nutrient solution with the addition of different concentrations of manganese chloride are shown as a parabolic relationship between toxic effect and concentration. Figure 27 shows two parabolas which represent McCool's results when peas are grown in soil having the same concentration of manganese chloride.[5] These results show a stimulation. The agreement of the experimental data with the curves are shown by Table 18.

[5] It is more than likely that the concentrations recorded here were not the concentrations available to the peas but are lower concentrations, due to surface adsorption of the manganese by the soil as was shown by Jensen (*8*) in his experiments with wheat.

TABLE 18.—*Comparison between the calculated and experimental values of the effect of manganese chloride on the growth of peas*

Concentration	Average length of tops		Concentration	Average length of tops	
	Calculated[1]	Observed [2]		Calculated[1]	Observed [2]
N	*Cm*	*Cm*	*N*	*Cm*	*Cm*
0	15.0	15.0	0.040	9.8	10.0
.002	16.0	16.0	.100	1.8	1.5
.020	12.6	12.5	.200	0	0

[1] Values obtained from the curves by adding the net effect to 100 per cent if the net effect is beneficial and by subtracting the net effect from 100 if the net effect is detrimental and multiplying the values by 15 (the normal growth or 100 per cent).
[2] From data by McCool (*12*).

McCool's data also supply some figures on the effect of calcium chloride on peas grown in distilled water. The data collected were entirely within the stimulating range. It is interesting to see how close the hypothesis of crossed parabolas will fit these data. Figure 28 shows the two parabolas chosen for this trial. Table 19 gives a comparison of the calculated and experimental results.

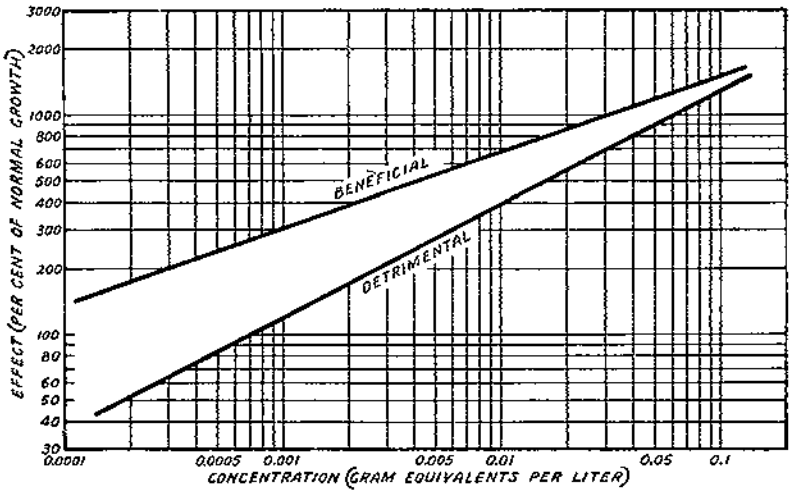


FIGURE 28.—Curves representing a beneficial and detrimental effect of calcium chloride on Canada field peas grown in distilled water. The data were collected entirely within the stimulating region. From data by McCool (*12*).

TABLE 19.—*Comparison of calculated and experimental stimulation of Canada field peas by calcium chloride*

Concentration	Weight of green tops after 30 days		Concentration	Weight of green tops after 30 days	
	Calculated[1]	Observed [2]		Calculated[1]	Observed [2]
N	*Grams*	*Grams*	*N*	*Grams*	*Grams*
0	1.85	1.85	0.01000	7.00	6.20
.10000	5.90	5.90	.00200	5.70	6.70
.04000	7.10	7.10	.00050	4.70	5.30
.02000	7.20	7.00	.00025	4.00	4.20

[1] Values obtained from the curves by adding the net effect to 100 per cent if the net effect is beneficial and by subtracting the net effect from 100 if the net effect is detrimental and multiplying the values by 1.85 (the normal growth, or 100 per cent).
[2] From data by McCool (*12*).

Only one of these values in Table 19 is as high as 1 gram difference between the experimental and theoretical results. The values therefore are certainly within the limit of measurement.

APPLICATION OF THE HYPOTHESIS TO FUNGI

At the time the experimental data shown in the early part of the bulletin were collected, no thought was given to possible stimulating effects; in fact, it was not known that timber-destroying fungi could be stimulated. It was not until the arrangement of many of the data in a form similar to the one presented that it became evident that some other influence must be at work to cause the discrepancies found in many of the data, and it was not until the idea of stimulation had been applied to Hopkins's data (p. 37) that the nature of the explanation became apparent. It was then too late to collect data on the stimulation of the test fungus. Since then Liese (*10*) has found that *Coniophora cerebella*, a wood-destroying fungus, is stimulated by dilute solutions of toxic materials. It seemed worth while, therefore, to review the experimental data on *Fomes annosus* for evidences of stimulations. Several instances of stimulation were found, some of them amounting to as much as 20 per cent. These data are much more liable to serious error than the data within the toxic range because the time during which they were growing seldom exceeded three days and never more than four and the measurements were probably not so carefully taken because they were of little interest at that time. Table 20 shows a comparison between the theoretical requirements for a pair of crossed parabolas and the observed experimental results.

TABLE 20.—*Comparison of calculated with experimental results of the effect of chrome alum on the growth of Fomes annosus*

Concentration	Radial growth		Concentration	Radial growth	
	Calculated	Observed		Calculated	Observed
Gram per liter	*Per cent of normal growth*	*Per cent of normal growth*	*Gram per liter*	*Per cent of normal growth*	*Per cent of normal growth*
0.014	128	132	0.11	3	1
.026	114	111	.14	0	0
.051	80	79			

It has now been shown that when the whole range of concentration is taken into consideration, the parabolic function must be replaced by two parabolic functions, one of which is beneficial and the other detrimental to the life of organisms, and that this relation seems to hold for bacteria, green plants, and fungi. It has also been shown that the two concepts of simple parabolic and crossed parabolic functions are compatible within the limits of the experimental error. Of the two, the concept of crossed parabolas is the more nearly complete since it covers the whole concentration range, whereas the simple parabolic relationship holds approximately only over a limited range. It is now desirable to determine whether the hypothesis of crossed parabolas can be applied to the effect of hyrdogen ions on the well-being of organisms.

APPLICATION OF THE HYPOTHESIS TO HYDROGEN ION

Investigations on the toxicity and stimulation of hydrogen ions are of interest to many fields from medicines to fertilizers because apparent small changes in the hydrogen-ion concentration may make great differences in the life of many organisms. These changes are so great that some investigators have postulated that all toxicity is founded upon the toxicity of hydrogen ions. Before the hypothesis of crossed parabolas can be applied to this type of work, it is necessary to discuss the differences between the work on hydrogen ions and work with any other ion.

(1) In all concentrations of hydrogen ions there are also present hydroxyl ions. The system of recording the concentration in pH values is such that even when the great preponderance of the ions present are not hydrogen but hydroxyl, the pH value is recorded in terms of the hydrogen ion. For the sake of clearness the number of hydrogen and hydroxyl ions in moles per liter is given in Table 21 for each whole number of pH. From Table 21 it is readily seen that a change in concentration of hydrogen ions from a pH value of 7 to a pH of 3 is not a small change but one of ten-thousand fold. A range of this magnitude is common in hydrogen-ion work. In dealing with hydrogen-ion concentrations two ions must be taken into consideration each of which may be toxic or stimulating, and at each concentration the effects of both ions must be present.

TABLE 21.—*Values of pH and their corresponding concentration of hydrogen and hydroxyl ions*

pH value	Concentration of hydrogen ions	Concentration of hydroxyl ions	pH value	Concentration of hydrogen ions	Concentration of hydroxyl ions	pH value	Concentration of hydrogen ions	Concentration of hydroxyl ions
	Moles per liter	*Moles per liter*		*Moles per liter*	*Moles per liter*		*Moles per liter*	*Moles per liter*
2	10^{-2}	10^{-12}	6	10^{-6}	10^{-8}	10	10^{-10}	10^{-4}
3	10^{-3}	10^{-11}	7	10^{-7}	10^{-7}	11	10^{-11}	10^{-3}
4	10^{-4}	10^{-10}	8	10^{-8}	10^{-6}	12	10^{-12}	10^{-2}
5	10^{-5}	10^{-9}	9	10^{-9}	10^{-5}			

(2) Though the neutral point of water as far as the electrical charge is concerned is at a pH of 7 when both ions are present in equal numbers, it is not necessarily the neutral point for fungi or bacteria any more than it is the neutral point for a large number of indicators. In fact, only the exceptional indicator correctly locates the ionic neutral point. Why certain organisms thrive best on slightly acid or slightly alkaline solutions is a very interesting speculation which need not enter into the discussion at this point, provided it is accepted that such is the case. It is only necessary to conceive that ionic neutrality is not necessarily neutrality toward any particular organism.

(3) Many fungi, bacteria, and other living organisms have the power to change the hydrogen-ion concentration of their medium, sometimes in one direction, sometimes in the other direction. Frequently this change is toward a definite pH which the organisms seem to prefer. This change is illustrated in Table 22, which is taken from the experimental data of Hopkins (*5*).

TABLE 22.—*Changes in hydrogen-ion concentration due to the growth of the fungus Gibberilla saubinetii on a liquid medium adjusted with sulphuric acid and sodium hydroxide* [1]

pH at beginning	pH after 7 days	Difference pH	pH at beginning	pH after 7 days	Difference pH	pH at beginning	pH after 7 days	Difference pH
3.85	4.4	0.55	5.5	5.5	0	7.4	5.6	1.8
4.8	3.6	1.2	5.8	6.2	.4	7.5	5.7	1.8
5.37	4.5	.87	7.5	5.2	2.3	7.6	5.9	1.7
4.8	6.0	1.2	7.5	5.2	2.3	8.25	6.9	1.3

[1] From data by Hopkins (*5*).

Curves produced by plotting growth figures (however expressed) against the pH value may be of many different patterns. The typical curve has two points of no growth—one at a high value and one at a low value of pH. As the pH increases from the low value at which no growth is obtained, the growth increases to a maximum point, then decreases to a minimum point, and then increases to a second maximum and finally decreases again to no growth at the higher pH value. Either of the maximum points may be higher than the other, and the minimum points may be either quite pronounced or scarcely noticeable.

In the previous discussion of the simple parabolic relationship and of the crossed parabolic relationship the basis of the calculation was the growth obtained in the absence of the poison. In hydrogen-ion concentration work it is not possible to obtain experimentally a growth in the absence of either hydrogen or hydroxyl ions.

A hypothetical normal growth, however, can be assumed. This assumption requires that the effect of both ions be referred to the hypothetical normal as 100 per cent normal growth. The effect of both ions will be represented by two effects—a beneficial effect and a detrimental effect—in exactly the same manner as has been done in the previous discussion, but with this difference: Each point on the curve, instead of being dependent upon a single detrimental and beneficial reaction, is in this case governed by four different reactions—a beneficial and detrimental reaction for the hydrogen ion and a beneficial and detrimental reaction for the hydroxyl ion. In other words, instead of a single pair of crossed parabolas, there are two such crossed pairs.

Inasmuch as the system of expressing hydrogen-ion concentration in terms of pH is a very convenient one and inasmuch as the pH value is the logarithm of the concentration, semilogarithmic paper may be used for plotting the curves. With semilogarithmic paper, the effect in terms of percentage of normal growth is plotted in logarithms, and the pH values are in ordinary units. Hopkins' work (*5*) on the fungus *Gibberella saubinetii* has been chosen for an example. This fungus is of great importance in agriculture, since it causes a serious disease of wheat and other small grains. Table 23 shows that this organism is capable of changing the hydrogen-ion concentration of the medium in which it grows. This change appears to be not toward the point of maximum growth but toward the minimum point. Changes of at least two pH units, which means approximately a hundredfold change in hydrogen-ion concentration, are given by Hopkins within seven days. (Table 22.) The pH value at the

beginning of the experiment is of no consequence, therefore, unless the solution were buffered. This Hopkins did in one series of tests. He records data for 4, 7, and 14 days. Even in buffered solutions the pH values are liable to change if the fungus is able to produce enough acid to use up the amount of buffer in the solution.

It might be expected, therefore, that even in buffered solutions some changes in hydrogen-ion concentration would be obtained. These changes would be more apt to be obtained in the longer period of time. Table 23 gives Hopkins' data on a buffered solution. In analyzing the data obtained after seven days a normal growth of 10

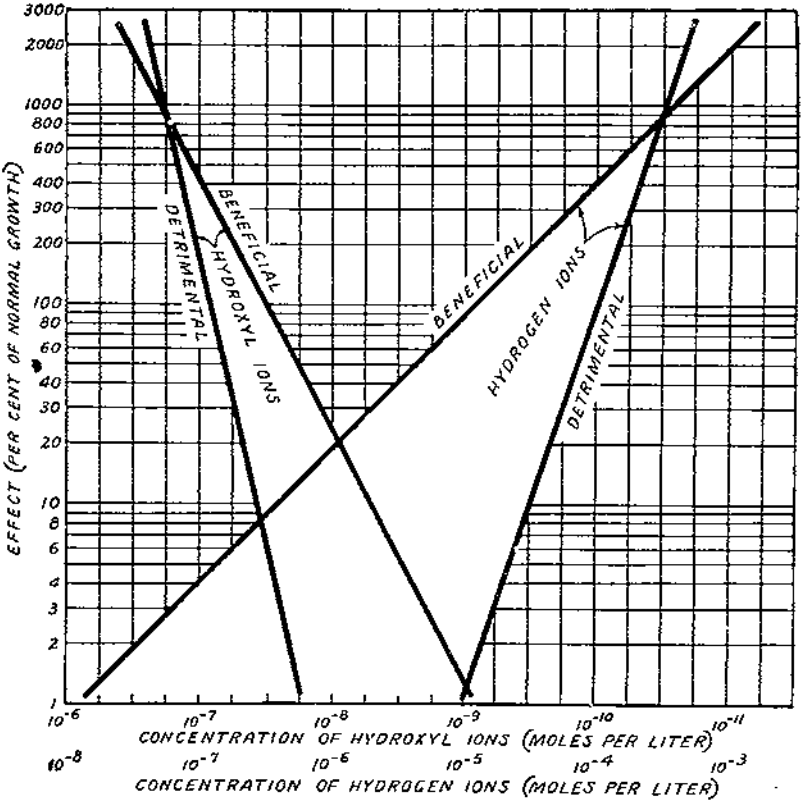


FIGURE 29.—Curves representing beneficial and detrimental effects of hydrogen and hydroxyl ions on the fungus *Gibberella saubinetii*. From data by Hopkins (6)

mg has been assumed, and all calculations have been based on this normal growth. Figure 29 shows the method of applying the analysis. Table 24 gives the net detrimental and net stimulating effect of each ion separately, also the total effect of both ions and the calculated total growth at the end of seven days. Figure 30 shows the calculated total growth plotted against the pH values in the usual way and a smooth curve drawn through the points thus obtained. The experimentally determined points have been added to show the accuracy with which the calculated curve fits the experimental points. Figure 31 shows the same kind of data for the 4-day period reported

by Hopkins (*5*). In this calculation the hypothetical normal growth was taken as 2. The four parabolas used in producing the two pairs of crossed parabolas were the same as were used in the previous example, with the exception of the beneficial effect of the hydroxyl ion. This lack of agreement seems to indicate that in the 7-day period some slight change in the hydrogen-ion concentration had taken place. This fact seems to explain why the same two pairs of parabolas can not be used to express the effect after 14 days. In the two periods shown the calculated results depict the experimental points well within the limits of experimental error. It seems, therefore, that the hypothesis of two pairs of crossed parabolas representing two beneficial effects and two detrimental effects—one pair for each ion—can be applied to hydrogen-ion toxicity work in exactly the same manner as it was applied to other ions.

TABLE 23.—*Hydrogen-ion concentration and growth of the fungus Gibberella saubinetii in liquid medium in which the reaction was adjusted with primary potassium phosphate, secondary potassium phosphate, phosphoric acid, and potassium hydroxide* [1]

Results after 4 days		Results after 7 days		Results after 14 days		Results after 4 days		Results after 7 days		Results after 14 days	
pH	Dry weight	pH	Dry weight	pH	Dry weight	pH	Dry weight	pH	Dry weight	pH	Dry weight
	Mg		*Mg*		*Mg*		*Mg*		*Mg*		*Mg*
2.80	0.0	2.77	0.2	2.80	2.2	6.05	3.0	6.85	28.2	7.15	71.1
2.90	.6	2.90	.8	3.15	3.6	7.10	7.0	7.10	48.8	7.45	66.1
3.05	0.0	3.10	1.8	3.25	17.2	7.10	10.6	7.15	31.7	7.55	73.2
3.40	1.8	3.47	3.8	3.85	70.6	7.10	2.8	7.20	13.6	7.70	47.8
4.25	6.0	4.60	47.4	5.00	42.2	7.15	7.8	7.20	3.2	7.75	70.8
4.75	4.8	4.60	23.4	5.40	70.4	7.15	.8	7.40	2.2	.....	.....
5.35	5.4	5.07	20.2	5.60	72.2	7.20	12.4	7.40	2.2	.....	.....
5.70	7.0	5.45	15.2	5.75	67.8	7.20	3.0	.....	.....	.....	.....
6.25	9.2	6.25	14.4	5.75	75.0	.....	.....	.....	.....	.....	.....

[1] From data by Hopkins (*5*).

TABLE 24.—*Separate effect of hydrogen and hydroxyl ions and total hypothetical effect obtained with the fungus Gibberella saubinetii*

pH	Effect of hydrogen ion [1]	Effect of hydroxyl ion [1]	Sum of the effects of the hydrogen and hydroxyl ions [1]	Total effect [2]	Calculated growth [2]	pH	Effect of hydrogen ion [1]	Effect of hydroxyl ion [1]	Sum of the effects of the hydrogen and hydroxyl ions [1]	Total effect [2]	Calculated growth [2]
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Mg*		*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Mg*
3.45	−140	.....	−140	−40	.....	5.50	+40	+6	+46	+146	14.6
3.48	−60	.....	−60	+40	4.0	5.75	+27	+11	+38	+138	13.8
3.50	0	.....	0	+100	10.0	6.00	+19	+24	+43	+143	14.3
3.55	+100	.....	+100	+200	20.0	6.25	+13	+47	+60	+160	16.0
3.60	+200	.....	+200	+300	30.0	6.50	+8	+92	+100	+200	20.0
3.75	+320	.....	+320	+420	42.0	6.75	+6	+172	+178	+278	27.8
4.00	+320	.....	+320	+420	42.0	7.00	+4	+250	+254	+354	35.4
4.25	+250	.....	+250	+350	35.0	7.10	.....	+200	+200	+300	30.0
4.50	+180	.....	+180	+280	28.0	7.15	.....	+100	+100	+200	20.0
4.75	+127	.....	+127	+227	22.7	7.25	.....	0	0	100	10.0
5.00	+86	.....	+86	+186	18.6	7.28	.....	−100	−100	0	0
5.25	+50	+2	+61	+161	16.1						

[1] In terms of normal.
[2] Total effect in percentage times normal growth of 10 mgs.

DISCUSSION OF THE HYPOTHESIS

Other investigators have explained the phenomenon of a minimum growth with two maximums in different hydrogen-ion concentrations on the basis of electrical charges and membrane equilibria, basing their work on the concepts of Loeb (*11*) and his associate and treating the hydrogen-ion work as special cases. Loeb (*11*) showed that when colloidal materials such as gelatin or mastic, which are amphoteric, are suspended in aqueous solutions, and an electrical potential is applied to the solutions, the suspended particles move toward one pole if the solution is of a higher negative potential than the suspended particles; toward the other pole if the solution has a less negative potential than the suspension; and remain stationary if the solution and suspended materials are of the same charge. This movement of the suspended particles is called cataphoresis, and the rate of movement is a measure of the difference in potential of the solution and the suspended particles. If the amphoteric substance is made in the form of a membrane and held rigid in solutions of different hydrogen-ion concentration, the same type of phenomenon takes place except that the solution moves from one side to the other according to the sign of the electric charge on the membrane, and the direction of movement can be changed by changing the hydrogen-ion concentration of the solution. This movement of water is called electrical endosmose, or electrical osmose. It, too, is a measure of the potential of the membrane. When nonliving materials, such as gelatin or mastic, are used, and the rate of movement is measured, the data form curves similar in many respects to the curves produced by living organisms in different concentrations of hydrogen ions. Donnan pointed out that the similarity of the curves suggested the importance of membrane equilibrium on the life of organisms. Other investigators following this suggestion have measured both the cataphoresis and the rate of growth of the same organism. For instance, Robbins (*14*) has shown that the isoelectric point for *Rhizopus nigricans*, a mold, is approximately at pH 5. He has also shown that the growth of the mold in a stated time has a minimum at pH 5.1, which is a very good agreement between the measurements of the physical characteristics and growth characteristics of the organism. Other investigators have obtained similar results with other organisms. In Figures 30 and 31 the minimum point shifted from a pH of approximately 5 in four days to a pH of 5.75 in seven days. It would be of interest to know whether the isoelectric point

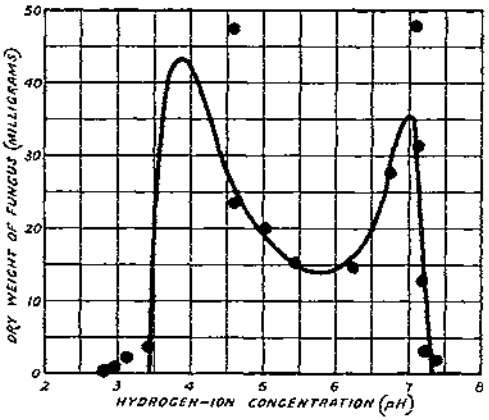


FIGURE 30.—Comparison of the hypothetical curve and the experimental results obtained with *Gibberella saubinetii* after seven days. The solid line represents the hypothetical curve, the dots the experimental data. From data by Hopkins (*6*)

also shifted and, if it did, what explanation could be given for this behavior.

Winslow, Falk, and Caulfield (*19, p. 197–198*) at the conclusion of their article on electrophoresis of bacteria, stated:

> Professor Donnan and other investigators have clearly understood the importance of applying the concept of membrane equilibria in the elucidation of physiological phenomena. Our findings add to the numerous vindications favoring this view and emphasize the importance of further study of membrane equilibria in bacterial suspensions. We have pointed out that certain potential differences between bacteria and their menstrua are apparently associated with some of the phenomena of viability. Viability and potential differences may, however, under certain conditions vary quite independently as evidenced by the fact that normal rates of migration are demonstrable after the cells have been killed by heat. Thus considerable caution must be exercised in relating the existence of these changes to the metabolism of the cell.

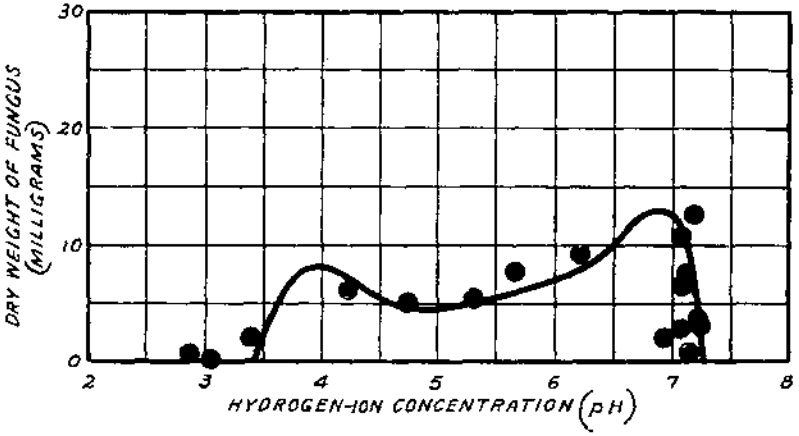


FIGURE 31.—Comparison of the hypothetical curve and the experimental results obtained with *Gibberella saubinetii* after four days. The solid line represents the hypothetical curve, the dots the experimental data. From data by Hopkins (*5*)

Loeb (*11*) showed that with suspensions of mastic, collodion, egg albumen, and gelatin the cataphoretic potential differences are the same for calcium, strontium, barium, magnesium, manganese, and cobalt. In fact he stated that, regardless of the chemical nature of the particles, the depressing influence of electrolytes on the cataphoretic potential difference is determined largely by the valency, but not by the nature, of that ion of an electrolyte which has a sign of charge opposite to that of the particle. It is well known, however, that a given concentration of calcium chloride will not have the same toxic effect upon living organisms as the same concentration of cobalt chloride although both have the same valence. Since, therefore, the toxic effect of salts, such as calcium chloride, can not be explained in terms of cataphoretic effect, why should this effect be expected to explain the toxic effect of different pH values—particularly when the same cataphoretic effect can be obtained with dead as with living organisms? It has been shown in this bulletin that the effect of hydrogen and hydroxyl ions on the well-being of the organism can be considered in exactly the same way as that of any other ions and that there is no need for a special case for hydrogen and hydroxyl ions.

Perhaps the concept of crossed parabolas also furnishes the clue (p. 42) to the reason why certain organisms have a minimum-growth point at one pH and others show a minimum growth point at some other pH. A change in the slope of any of the four parabolas will change the minimum point.

The similarity of the cataphoretic effect and the physiological effect in hydrogen-ion work can be explained on the following basis: Both effects are dependent upon the concentration of both the hydrogen or hydroxyl ions. Both would be a measure of the same thing and, therefore, changes in the hydrogen-ion concentration should, in general, give the same type of curve. There is this difference, however, that the physiological effect first shows an acceleration of growth followed by a rapid depression until no physiological effect can be produced at certain concentrations. Amphoteric membrances first increase their migration velocity and then decrease it, but there is no point except perhaps in very concentrated solution where the velocity is zero. Here at least is a difference in the effect caused by the same change in hydrogen-ion concentration.

APPLICATION OF THE HYPOTHESIS TO ANTAGONISM

Often when more than one chemical is used the toxic effect of the more toxic is offset by the presence of the other. This offsetting, or minimizing of the effect of one chemical by another, is known as the antagonism of one chemical for the other, or simply as antagonism. This antagonism is of particular interest in medicine and in agriculture; in the former because a poisonous effect of one material may be offset by the antagonistic effect of the other, and in the latter because the deleterious effect of chemicals may be offset by the action of other chemicals in the form of fertilizers. Part at least of the antagonisms might be explained on the assumption that, if one chemical were used at a concentration whose net effect was beneficial and the other at a concentration whose net effect was detrimental, the two effects would tend to neutralize each other. If then the effect of the two compounds were known independently, it might be possible to calculate the effect of both combined on the assumption that the combined effect would be the algebraic sum of their individual effects at the same concentration. McCool's work referred to on page 33 furnishes a number of results which might serve as a test for this idea.

Nowhere in McCool's results (*12*) is it possible to gather data showing the total effect of manganese chloride. Fortunately, however, McCool presents growth data for peas grown in different concentrations of both manganese chloride and calcium chloride, and also the growth data for the control. From these data the effect of any particular concentration of manganese chloride and calcium chloride can be calculated directly. For example, McCool gives the weight of the green tops of 10 peas in distilled water as 1.85 g, and in N/10 calcium chloride as 5.90 g. Taking the 1.85 g as representing the normal growth, the N/10 calcium chloride stimulated the growth 4.05 g, or 219 per cent. The growth obtained with N/4000 manganese chloride was 1.80 g; that is, a retarding effect of 0.05 g, or −3 per cent. The sum of the two effects if peas were grown on N/10 calcium chloride plus N/4000 manganese chloride should be 219 −3, or 216

per cent stimulation, or 4.01 g in excess of the 1.85 g normal growth. The expected growth is therefore 5.85 g. McCool found 5.76 g experimentally. Other data calculated in the same manner are given in Table 25.

TABLE 25.—*Comparison of the calculated and experimental results of the weight of the green tops of Canada field peas grown 30 days in mixtures of calcium and manganese chloride in distilled water*

Mixture	Calculated	Observed [1]	Difference
	Grams	*Grams*	*Grams*
N(0.1) $CaCl_2$+N(0.001) $MnCl_2$	3.94	5.12	−1.18
[2]N(0.1) $CaCl_2$+N(0.0005) $MnCl_2$	5.15	5.14	+.01
N(0.1) $CaCl_2$+N(0.00025) $MnCl_2$	5.85	5.76	+.09
N(0.04) $CaCl_2$+N(0.001) $MnCl_2$	5.25	4.52	+.73
N(0.01) $CaCl_2$+N(0.001) $MnCl_2$	5.10	5.58	−.48
N(0.002) $CaCl_2$+N(0.001) $MnCl_2$	4.35	3.75	+.60
N(0.0005) $CaCl_2$+N(0.001) $MnCl_2$	3.40	3.45	−.05
N(0.00025) $CaCl_2$+N(0.001) $MnCl_2$	2.40	2.01	+.40

[1] From data by McCool (*12*).
[2] Effect of this concentration of $MnCl_2$ estimated from curve of $MnCl_2$ alone.

The agreement between the calculated and experimental results given in Table 23 is fair when it is considered that each calculation may involve two experimental errors and that the experimental result may also contain some errors. Similar agreements with other pairs of chemicals can be shown from McCool's data, but these are sufficient to indicate that at times the antagonistic effect can be calculated from a simple consideration of effect produced by each chemical taken alone. McCool (*12*) also presents data on the effects produced by growing peas in nutrient solution containing different combinations of calcium chloride and manganese chloride. This should also be capable of calculation if the effect of the nutrient solution can be estimated. The nutrient solution was made up as follows: Calcium nitrate, 4 g; potassium monophosphate, 1 g; potassium nitrate, 1 g; magnesium sulphate, 1 g; potassium chloride, 0.5 g; ferric chloride, 0.1 g; distilled water, 3 liters.

Peas grown in distilled water produced green tops in 30 days that weighed 1.85 g. Peas grown on the foregoing nutrient solution produced 10.40 g of tops in the same time. The difference between the two weights represents a simulation of the solution of 8.55 g, or 462 per cent.

Since this is an attempt to calculate the antagonistic effect of calcium and manganese in nutrient solution and since the nutrient solution contained calcium, it is necessary further to factor the effect of the nutrient solutions. The concentration of calcium in the nutrient solution was N/0.016. In Figure 28 the stimulation of this concentration; that is, the difference between the beneficial and detrimental effects, is calculated as 300 per cent. The stimulation of the nutrient solution is about 460 per cent of the growth in distilled water, 300 per cent of this is due to calcium and 160 per cent is due to elements other than calcium or manganese. The figure 0.016 was added to the concentration of the calcium chloride used in nutrient solution to determine the total concentration of calcium.

The solutions of manganese chloride used in this experiment were much more concentrated than those in some of the previous experi-

ments of McCool, so that for the most part the effect of manganese chloride alone is unknown. This effect can, however, be calculated if it be assumed that the effect of manganese chloride in nutrient solution is made up of (1) a stimulating action of the solution and (2) an effect of the manganese. (Table 26.)

TABLE 26.—*Calculation of the effect of manganese chloride in a nutrient solution on the growth of peas*

Item	Grams	Percentage of normal growth
Normal growth of peas in distilled water	1.85	
Growth in nutrient solution	10.40	[1] 462
Growth in nutrient solution containing N/100 $MnCl_2$	2.25	[1] 22
Detrimental effect of N/100 $MnCl_2$		440

[1] Stimulating.

Values for N/50, N/200, N/500 manganese chloride have been worked out in a similar manner to that shown in Table 26. These taken with data obtained from McCool's experiments in distilled water were plotted on logarithmic paper to get the most probable effect in distilled water of manganese chloride at all ranges of toxic concentrations.

The total effect of calcium and manganese in nutrient solution is therefore made up of four factors: (1) Normal growth in distilled water; (2) effect of the chemicals other than calcium in the nutrient solution; (3) the effect of the calcium chloride referred to its effect in distilled water; (4) effect of the manganese chloride referred to its effect in distilled water. Each of these effects has been estimated separately. It is only necessary now to combine them. This has been done in Table 27.

TABLE 27.—*Comparison of calculated and experimental results of the weight of green tops of Canada field peas grown 30 days in different concentrations of calcium chloride and manganese in nutrient solution*

Concentration			Effect in terms of normal growth				Growth	
Calcium chloride		Manganese chloride, recorded	Calcium chloride	Manganese chloride	Nutrient solution except calcium	Total effect	Calculated	Observed [1]
Recorded	Actual							
N	*N*	*N*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Grams*	*Grams*
0.100	0.116	0.020	+200	−520	+160	−160	0	0.50
.100	.116	.010	+200	−420	+160	−60	.74	5.05
.100	.116	.002	+200	−180	+160	+180	5.18	7.35
.020	.036	.020	+300	−520	+160	−60	.74	.45
.020	.036	.010	+300	−420	+160	+40	2.59	2.55
.010	.026	.020	+300	−520	+160	−60	.74	.35
.010	.026	.010	+300	−420	+160	+40	2.59	2.55
.010	.026	.002	+300	−180	+160	+280	7.05	7.19
.002	.018	.020	+300	−520	+160	−60	.74	.37
.002	.018	.010	+300	−420	+160	+40	2.59	2.10
.002	.018	.005	+300	−300	+160	+160	4.81	3.15
.001	.017	.010	+200	−420	+160	+30	2.40	1.60
.001	.017	.005	+200	−300	+160	+150	4.62	2.10

[1] From data by McCool (*12*).

The agreement of the calculated with the experimental results is by no means perfect. It is believed, however, that all but one of them are within the ranges of experimental error and the error of calculation. The basis for the calculation was the weight of the green tops grown in distilled water. This was used to calculate the percentage effect of the nutrient solution. A variation in this basis would make an enormous variation in the calculated results. A variation from 1.85 g to 2.10 g in the weight of the green tops in distilled water would have made all the experimental results higher than the caluclated results and would have given negative growth for three of the calculated figures. McCool (*12*) does not give many determinations of the weight of the green tops grown for 30 days but he does give a number of such determinations after 24 days' growth. These are shown below. With so large a variation in the basis of calculation all but one of the 13 calculations in Table 27 would be considered as check results.

Experiment No.	Weight of green tops	Experiment No.	Weight of green tops
	Grams		*Grams*
1	1.72	5	1.85
2	1.70	6	1.70
3	1.62	7	1.65
4	2.20	8	1.75

It is therefore evident that some of the data on the antagonistic action of one salt for another can be explained entirely by a consideration of a stimulating and retarding action as presented here. The complete explanation is yet imperfect, just as the explanation presented by representing the toxic action by a simple parabolic function of the concentration was shown to be imperfect. The method of calculation presented here does not and can not explain the stimulating effect obtained by combining two compounds each in concentrations which alone would give a toxic action. In order to do this it seems necessary that the concentrations used in some way be reduced until at least one of the chemicals produces a stimulating action. Little is known about the concentration of chemicals within living membranes. Generally it has been supposed that these concentrations are the same as in the solutions upon which the organisms grew, or at least that the equilibrium between the concentration within the membranes is in some direct ratio to the concentration in the solutions. This equilibrium between the concentration on either side of a membrane might be upset very easily by the presence of another constituent. The theory of membrane equilibrium brought out by Donnan probably provides the basis for future extension of the work on antagonism. If the living membrane is amphoteric and contains ionized but nondiffusible compounds, or is capable of forming such compounds, then very different concentrations may exist in equilibrium conditions on either side of such a membrane. Calculations on the effect of such amphoteric membranes require a knowledge of the base combined with the membrane or the proportion of each base combined with the membrane if more than one could be combined at the same time. This information is not available. Analysis of the ash of roots of plants grown in mixtures of antagonistic compounds

should be of interest in this connection since it might give some idea of the ratio of the materials within the membranes in comparison with the ratio of the same materials outside the membranes.

SUMMARY AND CONCLUSIONS

The normal radial growth of the fungus *Fomes annosus* is directly proportional to the time of growth, provided other conditions are the same.

The radial growth of the fungus *Fomes annosus* is directly proportional to time even when poisonous materials are added to its food supply, provided other conditions are the same and the concentration of the poison is not lethal.

The change in the rate of growth of the fungus *Fomes annosus* due to the presence of a poison appears, within limits, to be proportional to a parabolic function of the concentration of the poison and can be expressed by the equation $R=DC^n$, in which R is the percentage retardation, C the concentration, and D and n are constants.

The constant D in the equation $R=DC^n$ may vary with each individual material, but the exponent n appears to be dependent upon the constitution of the poison. The exponent n has one value if the poison is an inorganic salt with a poisonous basic radical, another value if the poison is an ortho substituted benzene derivative, and still another value if it is a para or meta substituted benzene derivative.

The idea that toxic action can be expressed as a parabolic function of the concentration appears to be applicable, within limits, to other organisms, such as aphids, bacteria, and green plants.

Stimulation and toxic action can be regarded as different phases of a pair of opposed reactions.

The whole range of physiological effect of a poison can be represented by the difference between crossed curves both of which are parabolic functions of the concentration.

The physiological effect caused by changing the hydrogen-ion concentration can be represented by the sum of the effects produced by both hydrogen and hydroxyl ions each of which can be represented by the difference between two curves which are parabolic functions of their respective concentrations.

A partial explanation of antagonism, based on the hypothesis developed from the previous work, is offered.

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