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Fungicidal effect of triethylene glycol vapor on spores of *Penicillium notatum*

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FUNGICIDAL EFFECT OF TRIETHYLENE GLYCOL VAPOR
ON SPORES OF PENICILLIUM NOTATUM

by

Charles Bradley Ward, Jr.

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Food Technology

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TABLE OF CONTENTS

	Page
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	4
A. Early Use of Germicides in Air	4
B. Use of Glycols as Germicides in Air	5
C. In Vitro Studies with Glycols	12
D. Mechanism of Aerial Disinfection	13
E. Air-Sampling Methods and Instruments	27
F. Some Aspects of Microbial Air Sampling	36
III. MATERIALS AND METHODS	40
A. Experimental Chamber and Accessories	40
B. Mold Spores: Culture and Suspensions	57
C. TEG Determination	61
D. Experiments with Possible Decomposition Products of TEG	65
E. Typical Procedures and Calculations of Experiments	69
F. Viability of TEG-Exposed Spores	73
G. Miscellaneous Experiments	75
IV. RESULTS AND DISCUSSION	77
A. Performance of Equipment, and Properties of Spore Aerosols	77
B. Effect of Vapor Concentration	94
C. Effect of Temperature	96
D. Effect of Relative Humidity	101
E. Viability of Spores Exposed to TEG Vapor	120
F. Possible Decomposition Products of TEG	126
G. Effect on Dry Spore Particles	133
H. General Discussion	135
V. SUMMARY AND CONCLUSIONS	139
VI. LITERATURE CITED	143
VII. ACKNOWLEDGMENTS	153

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I. INTRODUCTION

Not until about 20 years ago did interest in air sterilization develop to any great extent. Interest was held in check for 35 years prior to this time because of a mistaken conception that air-borne transmission of disease was insignificant. In 1934 when it was demonstrated, with the aid of a new and efficient air-sampling device, that bacteria in dried air-borne droplets (droplet nuclei) might remain suspended almost indefinitely with the aid of slight air currents, interest in air sterilization developed.

Triethylene glycol (TEG)* vapor and also propylene glycol vapor have been found to be some of the most suitable germicides for air sterilization (Robertson, 1943b, pp. 227-254). Their effectiveness against viruses and against all vegetative bacterial forms tested has stimulated interest in these compounds.

The vulnerability of mold spores to glycol vapors has been but scantily examined. Mallmann and Churchill (1946), after examining the effect of several air sterilizing methods on microorganisms sprayed into food cold-storage houses,

*The abbreviation TEG will be used throughout this thesis.

reported that propylene glycol vapor was ineffective against the spores of Aspergillus niger and Penicillium italicum, while vegetative bacterial cells were reduced in numbers. Vapor concentrations and other important facts, however, were not reported.

Using an experimental chamber, Mellody and Bigg (1946, pp. 45-56) tested TEG vapor against Penicillium notatum spores. Their findings revealed that TEG vapor is fungicidal, but that concentrations about ten times as great as those required for bactericidal action are necessary for optimal killing. The fungicidal concentrations reported necessary amounted to several times the saturation capacity of the air.

A controversy regarding the mechanism of action of TEG and propylene glycol has appeared in the literature. One opinion attributes the removal of viable microorganisms from air by glycols to mechanical settling, whereas most investigators report that the glycol vapors have true germicidal action.

The dispute concerning the manner of action of glycol vapors appears to have aroused some doubt in the minds of certain food packagers as to the manner in which the vapor affects microorganisms with which they are concerned. It is the purpose of the present study to determine the extent of effectiveness of TEG vapors upon mold spores under

various conditions, and to investigate various aspects regarding the mechanism of action of the glycol vapors.

II. REVIEW OF LITERATURE

A. Early Use of Germicides in Air

The history of the use of germicides in air has been discussed by several authors, notably Robertson (1943a, pp. 499 to 502), Jennings et al. (1944), and Wells (1955, p. 59). Of interest in the history of aerial disinfectants was the use of glycols as hygroscopic vehicles to retard the evaporation of other germicidal aerosols. The term "aerosol" applies, in its strict sense, to stable suspensions of fine particles, solid or liquid, in air. Most workers studying the biological effects of air-borne particles have used the term in reference to suspensions of coarser particles which settle more or less rapidly. The writer will use the word in its latter sense in order to conform with the usage of these workers.

It was at first believed by almost all investigators except Masterman (1938, 1941) that the mechanism of action of germicides in air was via aerosol droplets (Twort et al., 1940, p. 339; Trillat, 1938; Pulvertaft and Walker, 1939). Therefore, compounds such as glycerol and glycols were added to germicidal solutions to retard evaporation of the aerosols.

A discussion of the presumedly-true mechanism of action of aerial germicides will be presented later.

A report by Robertson et al. (1941a, pp. 213 and 214) indicated that glycols themselves were effective bactericidal aerosols. Although other workers had used glycols and glycerine as hygroscopic vehicles, they had ascribed little or no importance to these compounds beyond their being useful as hygroscopic solvents.

B. Use of Glycols as Germicides in Air

The group under O. H. Robertson at the University of Chicago, working on air sterilization by glycols for more than 10 years, probably contributed more toward our knowledge of the subject than any other group of investigators. Following the discovery of the effectiveness of glycols as aerial germicides it was soon found that of several glycols tested, propylene glycol was the least toxic to animals and was also quite an effective air disinfectant. Therefore, early work on air sterilization by glycols was done with propylene glycol. After a period of 2 to 3 years, however, it was discovered that TEG was just as effective as propylene glycol and, in addition, an amount about 100 times less was

required for the same effect. TEG was also quite non-toxic in the amounts required.

The successful use of propylene glycol when introduced into the air of small spaces by means of atomization may be explained by its rapid evaporation to the vapor form. A relatively high vapor concentration of propylene glycol is liberated within a second or two after spraying into an aerosol mist (Robertson, 1943a, p. 501). Mather and McClure (1945) were unable to obtain bacteriocidal effects with propylene glycol when using a spray chamber in the ventilation system of a military barracks. However, when heat vaporization was added as a supplement, bacteriocidal effects were attained.

TEG does not evaporate fast enough from an aerosol to produce good killing under any normal conditions (Robertson, 1943b, pp. 233, 234). Some form of direct vaporization must therefore be used. This presents an engineering problem, since TEG decomposes before its boiling point is reached. Statements regarding the thermal decomposition point of TEG are at variance in the literature. For example, Gallagher and Hibbert (1937) have set the initial temperature of decomposition at 206.5°C , as measured by observing a rather abrupt change in vapor pressure with rising temperature. Others have reported decomposition temperatures without

giving the methods used for their determination. Lester et al. (1950, p. 816) reported

. . . no apparent decomposition of TEG when temperatures up to 290°F were maintained at the site of vaporization, provided the vaporizing unit was designed so that the heat was applied to the liquid TEG only at the point of vapor formation.

On the other hand, decomposition was frequently noted when the liquid TEG was kept above 120°F for long periods of time. Hamburger et al. (1945c) reported decomposition of glycols at 260°F. The precise nature of the decomposition products of glycols is unknown, but they may well be irritating or toxic and their formation should be avoided (Lester et al., 1950, p. 816).

Humans have been found to adapt to a prolonged moderate fog of TEG (e.g., in a hospital) without observed harmful effects (Hamburger et al., 1945b). However, humans entering a room having a fog of germicide, even though it may be odorless as appears to be the case with a slight supersaturation of TEG, generally react negatively. Robertson et al. (1947) exposed monkeys and rats continuously to high concentrations of TEG and propylene glycol vapors (some concentrations were greater than saturation) for 12 months or longer. No damage to any of the animals could be observed; the examinations included autopsy. In fact, rats breathing the vapors showed higher weight gains than the controls.

In laboratory experiments, concentrations of TEG just below the saturation of air, i.e., 3 to 5% per l of air at relative humidities of 15 to 40 per cent and temperatures of 72 to 76°F, were found to produce rapid killing of all pathogenic bacteria or viruses tested (Lester et al., 1949, pp. 180-182). Killing could be detected in a matter of seconds, and was complete in a matter of minutes.

The optimum range of relative humidity for bactericidal action has been determined as 25 to 60 per cent (Mellody and Bigg, 1946, p. 51) with a rapid drop in activity for conditions outside this range. The rate of bactericidal action has been found to increase with the relative saturation of the air with glycol vapor, at least up to the point of 100 per cent saturation of the air (Lester et al., 1949, p. 187). A certain minimum concentration of about 50 per cent saturation is necessary, however, in order to produce any appreciable germicidal action.

It has been shown (Bigg, 1943, pp. 120 and 121) that bacterial spores (Bacillus subtilis) are completely resistant to a saturated atmosphere of propylene glycol. Killing of mold spores (Penicillium notatum) has been reported by Mellody and Bigg (1946) to require supersaturated atmospheres of TEG, i.e., 10 to 5% per l of air at relative humidities of 45 to 65 per cent.

A complicating factor in the study of aerial disinfection is that aging of bacterial aerosol in the absence of any disinfectant modifies the lethal rate of survivors, possibly because of (1) selection of more resistant individuals, (2) biological change in the organisms (e.g., with desiccation), (3) formation of a protective, casehardened coat of foreign solids around the bacterial cell, or (4) a combination of all three. In dehydration, the chemical composition of the nucleus (the micro-environment) in air as well as the composition of the atmosphere (the macro-environment) must be considered. For example, Dunklin and Puck (1948, pp. 88-97) found that when the atomized bacterial suspension contained 0.5 per cent saline, broth, or saliva, pneumococci died rapidly in air alone (no disinfectant) at relative humidities near 50 per cent, while they survived fairly well at the humidity extremes. When the organisms were atomized from distilled water, lesser survival at 50 per cent relative humidity did not occur. It was postulated that there is a critical moisture content for bacterial droplets where concentrated solids are lethal.

Wells (1955, p. 57) reported decreasing survival of vegetative forms of bacteria upon increasing the relative humidity from 36 to 70 per cent. Speaking of the results of Dunklin and Puck, Wells (1955, p. 58) states:

. . . since these observers did not find the same critical mortality when salt was omitted from the aerosol, or with droplets of different size, deviations in lethal dehydration could arise from various factors in the microenvironment.

Wells (1955, pp. 60, 61) further asserts that, in general, the initial death rate of pathogenic bacteria is higher when they are sprayed into dry than into moist air, although the longevity of survivors seems to be greater in dry than in moist air. Moreover, Wells (1955, p. 54) lists several groups of workers who have reported results essentially in agreement with his own.

Dried bacteria from dust, wearing apparel, bedclothes, and other sources are known to be relatively resistant to TEG vapor (Robertson, 1947, p. 311; Robertson et al., 1951b). This makes it almost essential to supplement the use of TEG vapor (or propylene glycol vapor) with a program of dust and/or lint control (Loosli and Robertson, 1945; Loosli et al., 1947). Various types of oil treatment may be applied to floors and/or bedclothes. Depending upon the amount of activity in a room, oiling reduces viable microbes in the air from about 0 to 90 per cent or more.

Since glycol vapors are ineffective against microorganisms in large dust particles (Puck et al., 1943), one would not expect sterilization of surfaces by these vapors. Floors

and walls of experimental chambers have been found sterile, however, after treatment of bacterial aerosols with glycol vapors (Robertson et al., 1942a).

Continuous use of glycol vapors for air disinfection presents many engineering problems (Robertson, 1947, pp. 315, 316; Puck and Chaney, 1946) some of which may not have been solved satisfactorily for all situations. The vapors of glycols are heavy and require some means of dispersal throughout an air space. Continuous control of vaporization correlated with ventilation is necessary. Air conditioned rooms are indicated, but there is difficulty with condensation of glycols in the ducts. Relative humidity also must be controlled.

Glycol vapors have been tested for their ability to reduce the number of microbes in the air and for their ability to reduce the incidence of disease in spaces such as schools, military barracks, and office buildings (Bigg et al., 1945; Gilcreas and Read, 1955, pp. 767-773); Hamburger et al., 1945a; Harris and Stokes, 1945). In general, the conclusions have been (Wells, 1955, pp. 231-239; Gilcreas and Read, 1955, pp. 767-773) that while glycols will reduce the numbers of microorganisms in the air, the incidence of human disease is not altered appreciably in most cases. The relative importance of contact versus air-borne transmission of

disease is not known, but possibly must be considered here. In addition, "checkerboarding" (the protection of people from air-borne disease during 8 hours a day, while they are unprotected at other times, such as on bus rides to and from work) which has been used in many cases, possibly could not be expected to significantly reduce the incidence of air-borne disease.

Other tests of glycol vapors, such as those in poultry houses (Wisconsin Agricultural Experiment Station, 1952) may have failed to prevent spread of air-borne disease because of interfering factors such as excessive dust. Tests in dairy plants (e.g., Bennett and Nelson, 1954) may have proved negative because of the high humidities involved (54 to 90 per cent in the instance cited).

C. In Vitro Studies with Glycols

In vitro, the glycols have proved to be weak germicides (Robertson et al., 1948, pp. 124-137). Many bacteria will actually grow in broth containing 15 to 20 per cent glycol, but are rapidly killed at glycol concentrations of about 85 per cent. Spores of B. subtilis, however, were unchanged in viability after a 72-hour exposure in vitro to propylene glycol (Bigg, 1943, p. 121). Robertson et al. (1948) have

reported that for killing of vegetative bacterial forms in vitro, the higher the TEG or propylene glycol concentration (beyond a certain minimum where killing first occurs, i.e., about 40 to 50 per cent) the greater the killing, up to about 98 per cent. A minimum amount of water (about 2 per cent) was found necessary for killing. The bactericidal rate increased, in vitro, with increase in temperature from 60 to 90°F. To determine whether or not the in vitro action were reversible, cells exposed to 80 and 98 per cent concentrations of TEG or propylene glycol were washed in gelatin-Locke's solution three times, using centrifugation. From an original concentration of five billion cells, 8 to 10 cells were recovered after the first wash, 10 to 300 after the second, and 50 to 1000 after the third wash. For all practical purposes it was concluded that the reaction was irreversible. Mellody and Bigg (1946, p. 50) reported that spores of P. notatum were killed in vitro at an optimum concentration of 85 per cent TEG, the rate becoming less with higher concentrations.

D. Mechanism of Aerial Disinfection

According to Puck (1947b, p. 754) experimental results which are consistent with the theory that aerial disinfectants

operate through the medium of the vapor phase have been reported by several investigators (De Ome et al., 1944; Personnel of Navy Medical Research Unit No. 1, 1944; and Stone and Burnet, 1945). Baker et al. (1940), who previously postulated an aerosol mechanism of action, were able to verify the experiments of Robertson et al. (1941b, 1942b) which suggested that pure vapors containing no aerosol particles are rapidly lethal to air-borne bacteria.

A plausible explanation (Puck, 1947b, p. 754) for earlier observations may be that the experiments which gave rise to the aerosol hypothesis of germicidal action dealt largely with water-insoluble substances, mostly phenolic derivatives (cf., Twort et al., 1940, p. 262, 286). Such compounds, having little affinity for moist bacterial particles would not exhibit much tendency to condense on the particles until a concentration very near to or equal to the saturation point had been attained in the atmosphere. Moreover, most of the compounds employed by these workers possessed extremely low vapor pressures, and thus the difference between the concentration required for bactericidal action and the saturation point represents so small an amount of material that it might easily have escaped detection by the methods employed. These methods were often admittedly highly approximate (Twort et al., 1940, pp. 286, 287).

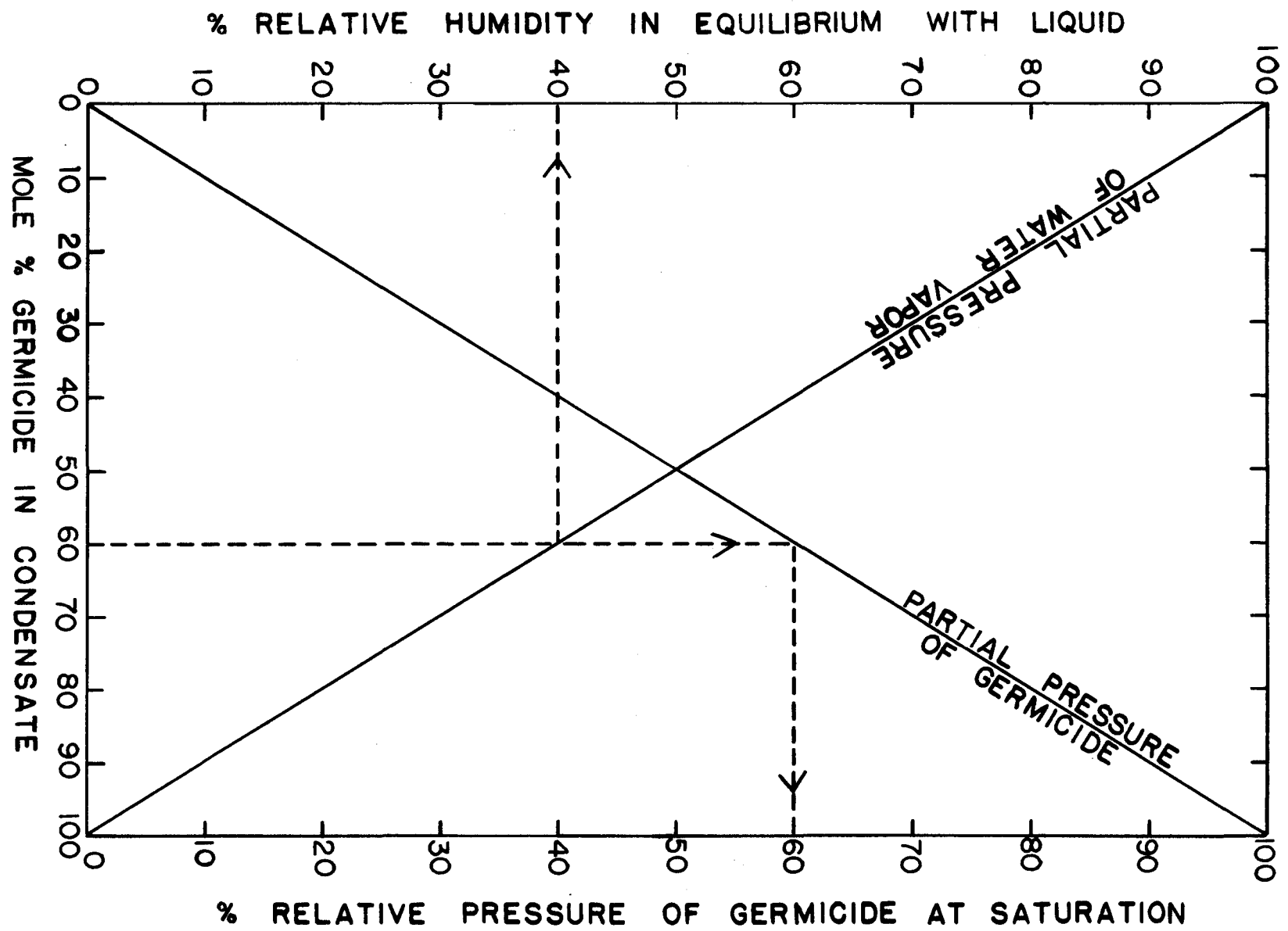
According to Puck (1947b, pp. 742, 743) concentrations of TEG, propylene glycol, dipropylene glycol, and ethyl alcohol needed to be disinfective *in vitro* are of the same magnitude, yet in air the concentrations necessary vary by more than 1000 times. All of these compounds are completely miscible with water and have the same chemical groups, but their effectiveness in air depends upon their vapor pressures. The lower the vapor pressure of the pure germicide, the lower is the concentration required for effective bactericidal action in air. Because of its lower vapor pressure, TEG is effective in lower concentrations than propylene glycol. However, the rate of action of TEG is slower because there are fewer molecules of vapor in the air to condense onto air-borne particles.

Puck (1947b, p. 748) has shown that Raoult's law defines the concentration of a completely miscible germicide in an air-borne water droplet for a two-phase, two component system. Thus, in air saturated with germicide vapor, water evaporates from and germicide vapor condenses upon air-borne particles (or vice versa) until the mole percentage of germicide in solution equals that present at equilibrium corresponding to the vapor pressure of the germicide at saturation.

Puck (1947b, p. 747) states that the presence of both a water-soluble disinfectant and water, in both liquid (droplet) and vapor states, allows prediction of concentrations by means of a phase diagram (see Figure 1). From the diagram, Puck has indicated that several points are evident: (1) The saturation concentration of the germicidal vapor decreases as the relative humidity increases. Note that at 40 per cent relative humidity condensation of germicide will theoretically not occur unless it is present at a pressure greater than 60 per cent of its pure vapor pressure, and the initial composition of the liquid condensate would be 60 mole per cent germicide and 40 mole per cent water. (2) Relative humidity controls the maximum concentration of germicide which the air can contain. That is, if the air were 100 per cent saturated with water vapor, no germicide could exist in the air. (3) In controlling the maximum concentration of germicidal vapor which can exist in the air, the relative humidity also determines the maximum concentration of germicide which can accumulate in air-borne, microbial droplets.

According to Puck (1947b, p. 750) glycol vapor can condense, because of its hygroscopicity, on air-borne particles even when its concentration in air is less than that required to saturate the atmosphere at the prevailing relative humidity. It is further stated that action of a germicide in air

Figure 1. Phase diagram (reproduced from Puck, 1947b, p. 748) showing the maximum relative percentage of the pure germicide vapor pressure which may exist in relation to the existing percentage relative humidity. The mole-percentage germicide in the condensing vapors is also given



depends, therefore, on its (1) hygroscopicity, (2) volatility, and (3) toxicity (high toxicity is apparently not necessary, but is desirable). TEG is quite hygroscopic, and has a low vapor pressure (0.0013 mm Hg at 25°C), both of which tend to cause its ready condensation.

As a result of his analysis presented in part above, Puck (1947b, pp. 752, 753) states that the lack of correlation of the phenol coefficient of a disinfectant with its air sterilizing ability may be attributed to its properties of hygroscopicity and volatility--properties of much greater importance in aerial disinfection than in liquid disinfection. These two properties were said to govern the physical phenomenon of condensation onto moist, air-borne, microbial particles such that a high concentration of germicide may rapidly develop in the particle from a relatively minute amount of vapor in the air.

The ineffectiveness of TEG vapor at high relative humidities has been explained by Puck (1947b, pp. 749, 750). He states that very little vapor can be maintained in the atmosphere at high relative humidities. Therefore, the concentration of germicide which can accumulate on moist air-borne particles may be so small as to be ineffective. A concentration of at least 50 per cent propylene glycol is required to produce rapid death of bacteria (Puck, 1947a).

The explanation Puck has given for ineffectiveness of glycol vapors in air of low relative humidity is that microbial particles may become almost completely desiccated and their surfaces may thus become resistant to condensation of hygroscopic vapor. Later, Dunklin and Puck (1948, p. 99) attributed the inefficacy to a combination of the reason given above and to the resistance of microorganisms, when desiccated, to many kinds of physical and chemical stresses. The latter fact is well known (Porter, 1946, pp. 195 and 196). Wells (1955, pp. 89-92), in order to demonstrate that the ineffectiveness of glycol vapors at low relative humidity was not caused by a lack of condensation onto bacterial particles, devised a simple but clever experiment. Measured quantities of the glycol (from 0.01 to 2 per cent by volume) were put into the bacterial suspensions used for atomization. These quantities had no noticeable effect in vitro, but provided for high initial concentration of glycol in the bacterial droplet when it was rapidly desiccated by being put into air of low relative humidity. The reason for the assumed concentration of glycol solution in the evaporating droplet is that the vapor pressure of TEG is quite low (0.0013 mm Hg at 25°C) so that its evaporation from the droplet is very much slower than that of the water. (Evaporation of airborne water droplets of a size of several microns is a matter

of only a fraction of a second at relative humidities around 50 per cent). According to Wells (1955, p. 100):

It was unnecessary during the period of observation to consider the absence of glycol in the atmosphere, since evaporation is a slow process for glycols of high boiling point.

It was assumed that only small amounts of glycol evaporated before disinfection should have taken place. When Wells conducted experiments such as described above he found that glycols were, as before, ineffective at low relative humidities. The conclusion drawn was that the invulnerability of organisms in droplet nuclei at low relative humidity depends upon biological changes induced by desiccation.

O. M. Lidwell (1948a, pp. 104 to 122) presents a scheme showing that at low relative humidity the rate of diffusion of germicidal vapor into the particle may be the limiting factor in the rate of kill, whereas at high relative humidity, diffusion through the gas phase may be the rate-determining process. This scheme was based on work with aliphatic, α -hydroxy carboxylic acids such as lactic acid.

At least three groups of investigators (Robertson et al., 1948, p. 136; Twort et al., 1946, p. 460; Grdn, 1950, p. 287) have assumed that the killing of microorganisms by germicides occurs by the same basic mechanism in liquid suspensions in the test tube or in moist particles in the air. Twort et al. (1946, p. 460) state:

With no knowledge to the contrary, it is assumed that the death of the microorganism is accomplished by the same mechanism under either condition [in air or in vitro], and that the same amount of germicide is absorbed in each case.

Since effectiveness as an aerial disinfectant increases in order, with ethylene glycol, diethylene glycol, and TEG (Lauter and Urla, 1940), it has been hypothesized that the ether linkage might be responsible for the increasing toxicity. In testing the toxicity of these compounds fed to rats, Lauter and his associates found TEG less toxic than diethylene glycol. Therefore, they state that it is doubtful that the ether linkage in higher glycols is responsible for the observed increase in effectiveness in air.

At constant relative humidity, temperature affects the action of glycols on air-borne microorganisms. Temperature rise has two effects (Puck, 1947b, p. 754): (1) it increases the vapor pressures of all compounds, and (2) it increases rates of all toxic actions. Puck further states that if the magnitude of the vapor pressure increase be greater, then temperature rise will lower the efficiency of any absolute concentration of vapor. This was found to be the case with glycols. Moreover, it was asserted that when the vapor pressure is increased, the concentration of glycol at equilibrium in the air-borne particle becomes less; however, if the same relative percentage saturation with vapor be

maintained along with the rise in temperature, killing action will be augmented.

Robertson (1943b, p. 246) has reported the following behavior of pneumococci left in propylene glycol for periods greater than a year. The autolytic enzyme system of the pneumococci was inhibited as long as the bacteria remained in the glycol, but when the cells were removed autolysis took place. Thus the autolytic enzyme system was only inhibited, not destroyed by propylene glycol.

Guided by a faint smell of formaldehyde in his vaporizing apparatus, Grün (1950, pp. 291, 292) tested TEG for traces of dissociation products. TEG gave a typical aldehyde color with Schiff's reagent, and the distillate from a TEG-water solution gave a positive test with chromotropic acid, a reagent specific for formaldehyde. It was stated that since formation of formaldehyde from TEG is plausible, the bactericidal effect of TEG may be ascribed to a formaldehyde component. The opinion was also expressed that TEG is superior to formaldehyde as an air disinfectant because the former compound is hygroscopic, thereby condensing on droplets suspended in space and quickly reaching a killing concentration.

Spores of bacteria are in no way affected by propylene glycol in vitro or in air (Bigg, 1943, p. 120, 121).

This fact, along with the known resistance of bacterial spores to desiccation, led Biga to support an hypothesis that the bactericidal action of glycols may be attributed to their hygroscopic properties. Since the glycols exhibit low toxicity in aqueous solution, this appeared to be an attractive hypothesis. However, glycerol, a highly hygroscopic compound of low vapor pressure, has little or no lethal power when vaporized into air containing bacteria. This fact would contradict the hypothesis of chemical dehydration.

A controversy regarding the mechanism of action of TEG and propylene glycol developed when Nagy and Mourmoseff (1950) of the Research Laboratories of Westinghouse Electric Corporation, Bloomfield, New Jersey, published a paper refuting the work of Robertson et al. (1941a, pp. 213, 214) of Chicago that glycol vapors are germicidal. The former investigators attribute the removal of viable microorganisms from air by glycols to mechanical settling. Although their work covered a period of several years, it was stated that they had not become interested in publishing the results until the University of Chicago research group designed, manufactured, and placed for sale a glycol vaporizer. Westinghouse and Company are manufacturers of a device employing an electrostatic means of removing particles from air. Evidence in

support of their own work has been given by Robertson et al. (1951a), and Lester et al. (1952). Nagy and Mouroumseff (1951) have continued to support their own belief. The details of this dispute are too numerous and complex to be dealt with in this thesis. However, some of the disputed topics will be mentioned in a later section.

Wells (1955, pp. 54 to 58) and his associates, have made an extended study of the lethal effect of atomizing bacteria into dry and moist airs. They found that bacteria put into dry air die rapidly within the first half minute, the rate slowing slightly or remaining constant thereafter. When bacteria were atomized into moist air, the death rate in the first half minute was small, and increased to the end of the 2-minute period, the total lethality being small in comparison to the corresponding dry-air experiment. Similarly-timed studies applied to bacteria atomized into rather moist atmospheres containing various glycol vapors revealed a pattern similar to that of the physical dehydration (evaporation) discussed above, i.e., the great majority of the killing took place almost immediately, after which the rate was considerably less. These phenomena show, according to Wells, a closer resemblance to the slackening death rate of physical dehydration than to the rather constant death rate of chemical disinfection of liquids or radiant disinfection of air (ultra

violet light). The decline in lethal rate was not uniform for all glycols, however, for with diethylene glycol and TEG the losses in the second minute were appreciable fractions of those in the first minute, indicating killing more consistent with a constant death rate. With respect to all the glycols tested, more effective killing was found with increase in either the number of hydroxyl groups, or the number of carbon atoms, or both.

It has been shown previously that the droplet nucleus--the microenvironment of the air-borne microorganism--is sensitive to the composition of the atmospheric macroenvironment in which it is suspended. Within the droplet nucleus, changes in equilibrium between gaseous, liquid and solid states, corresponding to physico-chemical atmospheric changes, might readily account for biological conditions within the droplet nucleus which affect the viability of the microorganism (Wells, 1955, p. 101).

The ineffectiveness (for all practical purposes) of glycerol as an air disinfectant, presents a glaring contradiction, Wells admits, to any simple interpretation of chemical dehydration as being the mechanism of action for the glycols. Therefore the following was written (Wells, 1955, p. 89):

We have not yet been able to formulate a consistent hypothesis to account for all these interesting

relationships and must therefore be content with a convenient descriptive analogy to physical dehydration.

Moreover, Wells (1955, p. 101) concludes that:

Whatever our attitude toward generalizations that are merely conveniently descriptive, we must recognize that this important new field of aerobiology cannot be deduced from conventional knowledge of microbiology.

E. Air-Sampling Methods and Instruments

Luckiesh and Taylor (1947) made the comment that bacterial air sampling is not an exact science. One can readily believe this statement when he reads the literature concerning comparisons of efficiency for microbial air samplers. There is disagreement among all groups of workers. However, since about 1945 there has been good progress toward the realization of factors which must be controlled in order to obtain adequate comparisons among microbial air samplers.

According to Lester (1955) no "foolproof" technique exists for the uniform evaluation of bacterial air samplers. However he has suggested procedures which he deems necessary for the evaluation of bacterial air samplers. These procedures are summarized here as follows:

1. To obtain the essential, bacterial aerosol of reproducible, uniformly-sized particles in an

experimental chamber, it is necessary to control (a) temperature, (b) relative humidity, (c) conditions of atomization, (d) chemical constituents used in the culture medium and atomizing vehicle, (e) rates of air flow, (f) position and site of sampling, and (g) metabolic and functional status of the biological agents used.

2. Use of a dynamic experimental chamber (having continuous air flow and continuous atomization of bacterial aerosol) rather than a static chamber is recommended, since in the latter there is interference from local variables.
3. Collection and retention efficiencies (collection efficiency refers to the organisms, both counted and uncounted, which are trapped in the sampling device, while retention efficiency refers only to the organisms which are counted) should be determined using an inert, non-volatile, stable aerosol such as a dye. These efficiencies should be determined for various rates of air flow, and over different ranges of particle size.
4. Whatever tracer is used (e.g., oil-soluble dyes, specific chemical tracer, radioactive agents) it is important that the technique is capable of

accurate, quantitative measurement in low concentrations and that the substances used are uniformly distributed throughout all particles in the aerosol.

5. Determinations of efficiency should be made on both wet and dry aerosols.
6. Studies should also include collection and retention efficiencies for biological aerosols of bacterial cells because factors present in sampling devices can influence survival of bacterial cells.
7. Because of the profound effect of relative humidity on the size of bacterial aerosols, one should correlate efficiency of any sampler with various relative humidities.
8. The same ranges of particle-size distribution advised for inert aerosols should be studied with bacterial aerosols.

From inspection of the above list, one can understand why completely adequate comparisons of microbial air samplers have not been made, and also why there is disagreement as to which sampler is most efficient for any given purpose.

Use of sampling devices for determining numbers of microorganisms in the air usually involves two basic operations: first, the separation of the organisms from a measured amount of air, and second, the determination of the number of

organisms separated. Since air-borne microorganisms are suspended in the same manner as dust particles or may be attached to the dust particles themselves, methods for sampling air-borne microorganisms closely follow those for dust in many instances.

In the separation of microorganisms from air the sampling principles used may be divided into these general types: (1) impingement of the organisms on adherent, solid or semisolid material, (2) entrapment of organisms by bubbling the air through a liquid, i.e., impingement in a liquid, (3) impingement with the aid of electrically-charged plates, (4) filters, and (5) precipitation of organisms with liquid spray or condensed steam.

Since microorganisms are ordinarily present in air in relatively small numbers, rarely more than a few hundred per cu ft, it is practically impossible to count them by direct optical methods. In nearly every case, therefore, the microorganisms are enumerated by their growth on a suitable culture medium. Direct, photoelectric counting, making use of light scattering by microbial particles has been used, but is strictly a laboratory method (cf. Ferry et al., 1949).

For a discussion of the air-sampling methods developed prior to 1933 one may consult Ruehle (1915), or Committee

on Apparatus in Aerobiology (1941). Only a few of these earlier methods will be discussed in this section.

Rettger (1910) developed an aeroscope (an apparatus for collecting dust, spores, bacteria, etc. from air suspensions), the use of which consisted essentially of bubbling air through liquid by means of a glass tube having an enlarged, perforated end. Aeroscopes of this type are used today.

Exposure of nutrient media in petri dishes for given lengths of time is one of the simplest of the impinging methods for microbial examination of air. This technique has undoubtedly been used for many years. In using this "settling plate" technique to obtain quantitative information, one must realize that (1) settling plates are selective for large particles which settle relatively rapidly from the air, and (2) the plates will not give reliable results in ordinary, occupied rooms because of non-uniform air eddies, and therefore are useful only in rigidly-controlled experimental atmospheres, and mainly as a supplemental technique in combination with other sampling devices capable of rapidly removing small particles from air.

The development (Wells, 1933, pp. 58, 59) of the air centrifuge, an instrument capable of quantitatively sampling small, air-borne particles, and the demonstration (Wells, 1934; Wells and Stone, 1934) of viable air-borne "droplet

nuclei" evoked a renewed interest in air-borne microorganisms. The air centrifuge spins microorganisms from the air onto agar or broth coating the inside of a glass tube. Since the air centrifuge was the only microbial air sampler commercially available for many years, it was extensively used, especially in this country.

Hollaender and Dalla Valle (1939) constructed a simple device usually referred to as a "funnel". A glass funnel is fixed in an airtight container so that when suction is applied to the container, air is drawn through the stem of the funnel and over the surface of an agar medium in a petri dish, thereby impinging microorganisms on the agar.

Bourdillon et al. (1941, pp. 197-224), at the National Institute for Medical Research, London, developed an apparatus, called a "slit sampler", for collecting and counting air-borne microorganisms. In this device a petri dish containing nutrient agar is placed on a disc capable of very slow rotation. The rotating disc and agar dish are sealed in a metal enclosure and air is drawn at high velocity through a narrow slit placed over a radius of the petri dish thereby impinging microorganisms from the air onto the agar.

Steaming is rather commonly employed for removing bacteria and dust from air inside hoods and other confined spaces. Elliott(1941) described a method for microbial air

sampling which used condensation of water onto particles thereby precipitating them in a chamber of about 100 per cent relative humidity. Humidification was obtained by means of a steam jet.

A device usually referred to as the "Moulton Air Sampler" (Moulton et al., 1943, pp. 51, 52) uses the principle of atomization to coat air-borne, microbial particles with a layer of liquid. Should the particles in the atomizing chamber fail to be trapped in the refluxing liquid, there is a second trap which bubbles the air through a second liquid reservoir.

A sieve device for sampling air-borne microorganisms was described by duBuy and Crisp (1944, pp. 829-832). The impinging principle is used in this apparatus which consists of two parts, a metal container which holds a petri dish with agar, and a metal top perforated with 341 small openings which is clamped onto the container. Upon application of suction to the device, air enters through openings of the sieve plate and impinges microorganisms onto the agar. The sieve device will be further discussed in the section on Materials and Methods.

A group of physicists (Luckiesh, et al., 1946), concerned by the shortcomings of the various microbial air samplers, developed an electrostatic air sampler. This instrument,

called the "Duplex Electrostatic Air Sampler", is essentially a dual "funnel" device having a positive, direct current potential of 7,000 volts applied to one petri dish, the other plate having a corresponding negative charge. Charged microbial particles are trapped on the plate of opposite charge.

Rosebury (1947, pp. 108-116) developed a microbial air sampler called a "capillary impinger". The device operates by impingement in a liquid, as with many bubbler samplers. A short capillary tube through which the air sample passes is submerged in the collecting liquid, the capillary tube serving also as limiting orifice for the air-flow.

Sonkin (1950) published a study on the use of a modified "Cascade Impactor" (May, 1945, pp. 187-195) as a microbial air sampling device, although this device was designed primarily for determining the size-distribution of aerosols, and has been quite often used as such (Rosebury, 1947, pp. 57 and 58; Dunklin and Puck, 1948, p. 88) by those studying biological aerosols. The modified "Cascade Impactor" consists essentially of a system of five jets and sampling slides in series. The jets are progressively finer, so that the air speed, and therefore the efficiency of impaction upon the slides, increases from slide to slide. For determining

numbers of air-borne microorganisms the organisms are washed from the impaction slides.

Recently, the molecular filter (commonly abbreviated "MF") has been adapted to sampling of air-borne microorganisms (Goetz, 1953, pp. 150-159). The Aerosol Assay types of MF are dry gels (a cellulose ester gel) of micellar structure, i.e., the filters are devoid of fibrous structure, being held together by intermolecular forces. The pore diameter has no direct relation to minimum particle size retained since the pores are slit-like rather than circular. In addition, large electrostatic forces which develop throughout the MF when air is forced through, hold particles smaller than pore size. The MF is rigid enough for handling, but must be supported during sampling. Supports are mounted in a device allowing the application of a vacuum to one side of the filter. After an air sample, the collected organisms are incubated in place on the MF disc which is placed in a sterile covered dish containing suitable nutrient solution for keeping the MF disc moist.

A very simple technique for microbial air sampling has been described by Griffin et al. (1956). The technique is called "Syringe Dilution". In this method, 5-ml samples of bacterial aerosol are withdrawn from a chamber, using 10-ml syringes containing 5 ml of 0.85 per cent saline. The

syringes have stopcocks to prevent leakage while being shaken for 1 min. Dilution and plating of the suspension then follow.

F. Some Aspects of Microbial Air Sampling

According to duBuy et al. (1945, p. 38) sampling into liquid media tends to break dust particles or clumps of microorganisms taken from the air, while sampling onto agar allows counting of each particle as a single unit, regardless of the number of viable organisms each particle may contain. It was stated that this inherent difference between the two methods tends to make sampling into liquid appear more efficient than it truly is in comparison with sampling onto agar.

Bourdillon and Lidwell (1948a, pp. 33, 34) list the following considerations as being of dominant importance in tests of sampling efficiency:

1. Size of the particles to be collected.
2. Inlet tube of the sampler--aperture, length, diameter, radius of bends, if any, and their number, smoothness of walls.
3. Configuration of jet orifice.
4. Velocity of air stream.

5. Distance from inlet orifice to collecting surface.
6. Rigidity and adhesiveness of the collecting surface and the return of the liquid surrounding it, if a liquid is used.
7. Relative humidity of the atmosphere immediately upstream from the collecting surface. (Cooling from adiabatic expansion may be sufficient to cause deposition of water on particles, and hence increase the ease of impingement).

From their extensive experience in air hygiene, Bourdillon and Lidwell (1948a, p. 33) give recommendations of the most suitable microbial air samplers to use for different size-ranges of particles. Their recommendations, along with their reasons, are summarized below.

1. For very large particles (100μ and upward) open dishes of agar or broth are probably the best. Slit and sieve devices are likely to become inefficient because of impaction of particles on the upstream side of the orifice plates. The large entry ports and low air velocities practicable with electrostatic air samplers give them special advantages for very large particles.
2. For large particles (100 to 30μ) a rather similar order of merit would be expected. Open dishes are

still useful, with the advantage possibly inclining towards the funnel, bubbler, Moulton device, and the air centrifuge.

3. For particles between 30 and 10μ , which are important in normal air sampling, most samplers should show reasonable efficiency. Open dishes, used alone, are of little value.
4. For small particles (10 to 1μ), also of great importance in normal sampling, open plates are of restricted value. Low velocity impingers, such as the Wells Air Centrifuge and the funnel device, are relatively less efficient than the high velocity impingers such as the slit samplers, the sieve device, the Moulton sampler, and the bubblers.

Bourdillon and Lidwell (1948b, p. 50) state that they know of no reason why broth samplers should be inherently more efficient than agar samplers or vice versa. Wells (1955, p. 40) states that:

In air washers [bubbler type samplers] the chance of [a particle's] colliding with liquid surfaces is apparently less dependent upon momentum. Exactly what governs the entrapment of particles by liquids has not been defined; it may well be that surface attraction depends upon the composition of the particle.

Comparisons of efficiencies of various microbial air samplers under various conditions have been reported by

several groups of workers (Bourdillon et al., 1941, pp. 212-214; duBuy et al., 1945, pp. 4-40; Rosebury, 1947, pp. 108-116; Goetz, 1953; Wells, 1955, pp. 35-40). Wells (1955, p. 38) has evaluated the results of several reports. It was found that the two most efficient instruments for sampling either (1) fine droplet nuclei or (2) coarse droplet nuclei were the slit sampler (Bourdillon et al., 1941, pp. 197-224) and the air centrifuge (Wells, 1933, pp. 58-59). The molecular filter (Goetz, 1953, pp. 150-159) only recently adapted to sampling microbial aerosols, was not included in the above evaluation.

Standard bacteriologic procedures for air sampling have been established by the American Public Health Association (Wells et al., 1946, p. 330). The two official instruments for sampling bacteria-bearing dust particles are the slit sampler and the air centrifuge, while for sampling total numbers of bacteria air washers (bubblers) and the air centrifuge using collection into liquid are official.

III. MATERIALS AND METHODS

A. Experimental Chamber and Accessories

1. Chamber

Studies of air-borne mold spores were conducted in a cube-shaped chamber. The internal height was 3.25 ft, and the volume, with devices inside, 31.4 cu ft. Photographs of the chamber may be seen in Figures 2 and 3. Four of the sides were made of 3/16-in pressed board, the other two being of double-walled plate glass. Insulation was glued on the board sides where convenient. The door to the chamber (Figure 3) was about 2½ ft square and fastened against a rubber gasket by means of wing nuts. Tests for airtightness were made with ammonium chloride smoke.

The chamber was fitted on top with a U-shaped air-cooling system (Figure 4) consisting of a 100-cfm blower, refrigerated coils, and polyethylene-fiber air filter. Two small airtight doors swung by hinges and capable of being operated from outside the chamber separated the air-cooling ducts from the experimental chamber.

Waterproof spar varnish (Kyanize brand) having exceptional resistance to chemicals was used to coat the inside

Figure 2. Photograph of the experimental chamber and some of the equipment used for aerial disinfectant studies

Figure 3. Photograph of the experimental chamber showing support on two timbers

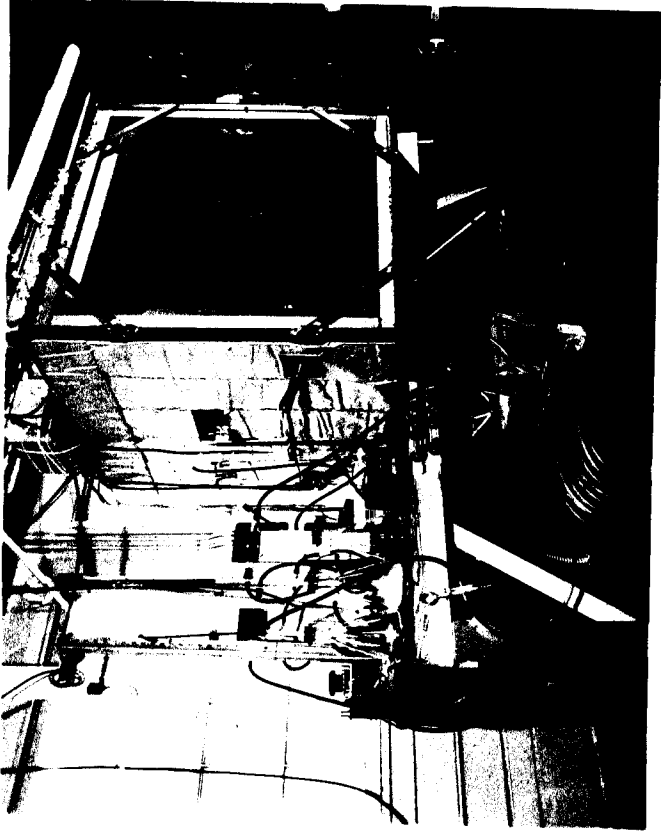
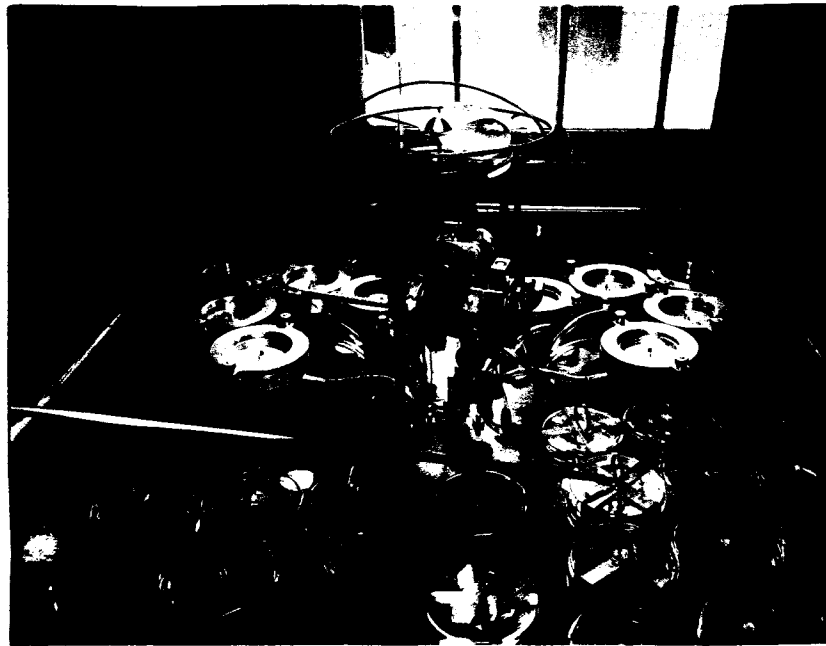


Figure 4. Photograph of the top of the experimental chamber showing insulated air cooling ducts including blower at left, refrigerated coils at center, and air filter at right

Figure 5. Photograph of floor of experimental chamber showing fan, with vaporizer beneath, petri dishes, and sieve air samplers at the far end. The rod in the foreground was used for removing petri-dish lids



walls of the chamber except for the glass. Mounting of the chamber on two, 2- by 4-in pieces of wood allowed insertion of tubing and other apparatus through the floor of the chamber.

To facilitate use of psychrometric charts, all temperature measurements relative to the chamber were recorded in °F.

2. Fans

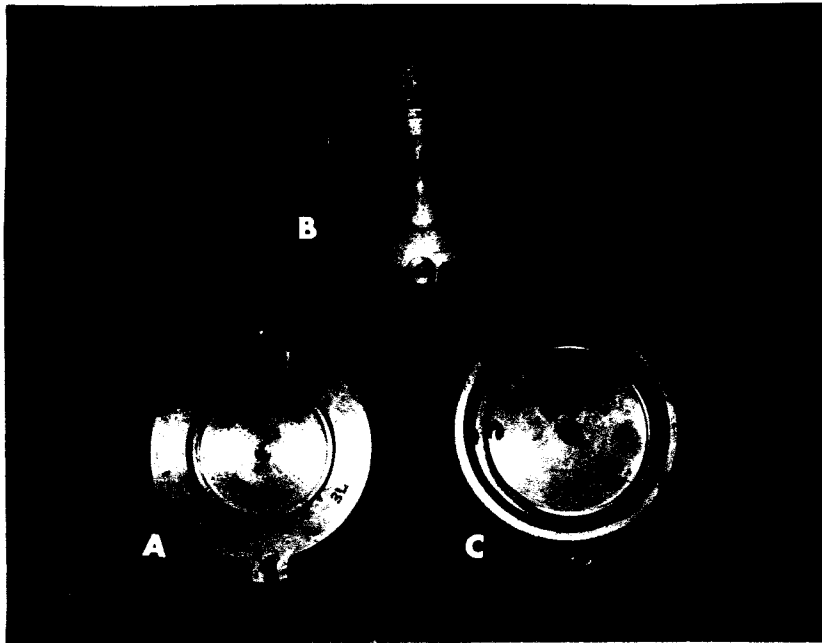
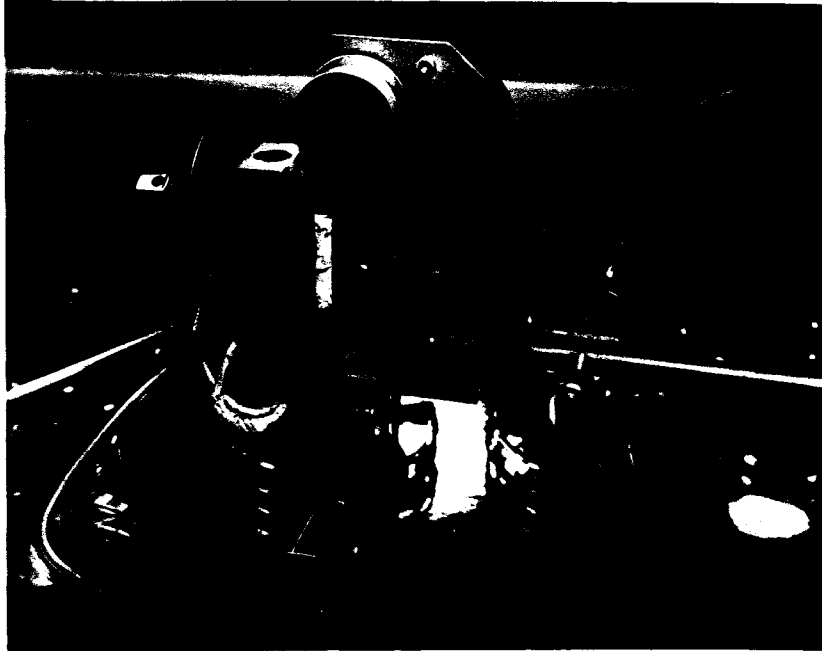
An 8-in fan, operated at 70 per cent of its stated voltage requirement, was mounted on a tripod in the center of the chamber (Figure 5). This fan was used to provide a draught for the vaporizer located immediately below it, and also to help maintain a uniform mixture of the atmosphere. In some experiments with vapors of compounds other than TEG, an 8-in fan with a long shaft running to a motor located outside the chamber was used.

3. Vaporizers

For experiments with TEG parts of a commercial vaporizer (Glycoaire) were used (Figure 6). This apparatus consisted of a woven glass belt held by two pulleys, one of which was driven at 1 rpm by a small electric motor and gears. The glass belt passed close to a heating block seen just to the left and below the top pulley in Figure 6. Beneath the

Figure 6. Photograph of the vaporizer used. Note glass tape running over two pulleys, and heating block to the left and below the top pulley. Reservoir is beneath the lower pulley

Figure 7. Sieve air-sampling device (duBuy and Crisp, 1944, pp. 829-832). "A" is sieve top which mounts onto "C" which contains petri dish. "B" shows the assembled sampler



lower pulley a stainless steel cup, mounted on a shaft movable in a metal sleeve, was used to contain liquid germicides. The cup, equipped with a lid, could be raised from beneath the chamber at the time it was desired to start vaporization, and thus submerge the belt in the liquid. The temperatures at the point where the wick passed the heating block were determined by means of a thermocouple.

At times, another vaporizer consisting of a stainless steel plate with a slight depression in the center was used. A thermocouple was silver-soldered in the depression in order to allow temperature determinations of liquid germicides on the plate. The plate when used was placed beneath the central fan.

4. Air samplers

The sieve sampler of duBuy and Crisp (1944, pp. 829-832) was one of the air-sampling devices used (Figure 7). This apparatus, made of Duralumin alloy in this instance, consists essentially of two parts, a container which holds a standard petri dish, and the sieve which may be clamped onto the top of the container and over the petri dish. In Figure 7, "C" is the container with a petri dish in place upon three spacer pins allowing air to be drawn beneath the petri

dish, through the hold in the center of the container and out the tube at the far right. The part labeled "A" is the top of the sampler and contains the sieve plate drilled with 341 very small holes (no. 79 drill) equally spaced. "B" is the assembled sampler with two bolts clamping the top against a rubber gasket. The sieve plate may be adjusted up or down over agar in the petri dish by screwing the sieve plate with a key. For determining the height above the agar there is a free-moving plunger (not discernible in the photograph) which rests upon the agar and indicates height on a scale at the center of the sieve plate. In Figure 5, ten of the sieve samplers may be seen assembled for air sampling.

The variability among ten sieve samples was analyzed by taking ten, 10-sec samples of air-borne mold spores at intervals of 1 min from an atmosphere of 48 to 52 per cent relative humidity. The mold spores had been sprayed into the chamber with atomizer B (described later) 4 min before sampling commenced. Colony counts obtained were converted to the common logarithms and adjusted to represent deviations from a common regression line by using the formula

$$\text{adjusted } Y = Y - bx$$

(Snedecor, 1946, pp. 114-117), where b is the slope of the regression line and x is the difference of an X value from

the mean of the X values. The standard deviation of the adjusted Y values (the Y values being the logarithms of the spores per cubic foot of air) was reconverted to the anti-logarithm.

Figure 8 pictures a modified Moulton air sampler (Moulton et al., 1943, pp. 51, 52) showing, from left to right, atomizing chamber, bubbling chamber and Kjeldahl trap. This sampler was used in early experiments.

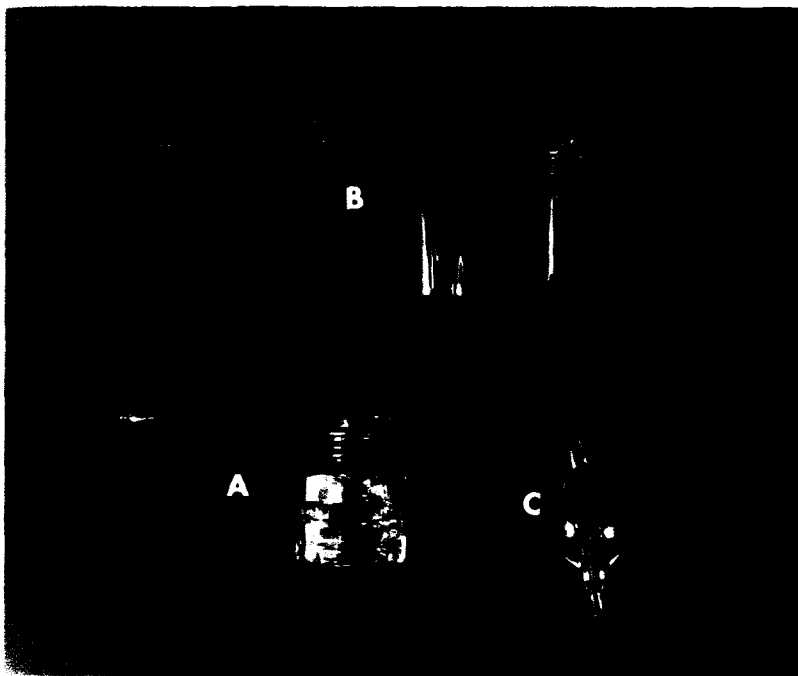
Settling dishes were exposed in sets of two or three. The dishes were opened and closed from outside the chamber by means of a sliding brass rod mounted in a ball and socket joint. This "artificial arm" may be seen in Figure 2 and in Figure 3. Ringed clamps for the dish lids may be seen in Figure 5. The arm was also used for lifting the lid from the reservoir of liquid germicide.

5. Relative humidity determination

Percentage relative humidity in the chamber was determined by means of dry- and wet-bulb thermometers read through the glass walls of the chamber. Thermometer readings were converted to relative humidity by means of a psychrometric chart. The cloth wick for the wet bulb was washed before each experiment. A portion of the relative humidity apparatus, which is operated by means of an electric fan, can be seen in Figure 5 at the far side of the chamber.

Figure 8. Photograph of a modified Moulton air sampler (Moulton et al., 1943, pp. 51, 52). Visible from left are the atomizing chamber, bubbling chamber, and Kjeldahl trap

Figure 9. Photograph of three atomizers used. "A" is a De Vilbiss-brand atomizer. "B" is a specially-modified De Vilbiss atomizer, and "C" is a De Vilbiss No. 44 nebulizer



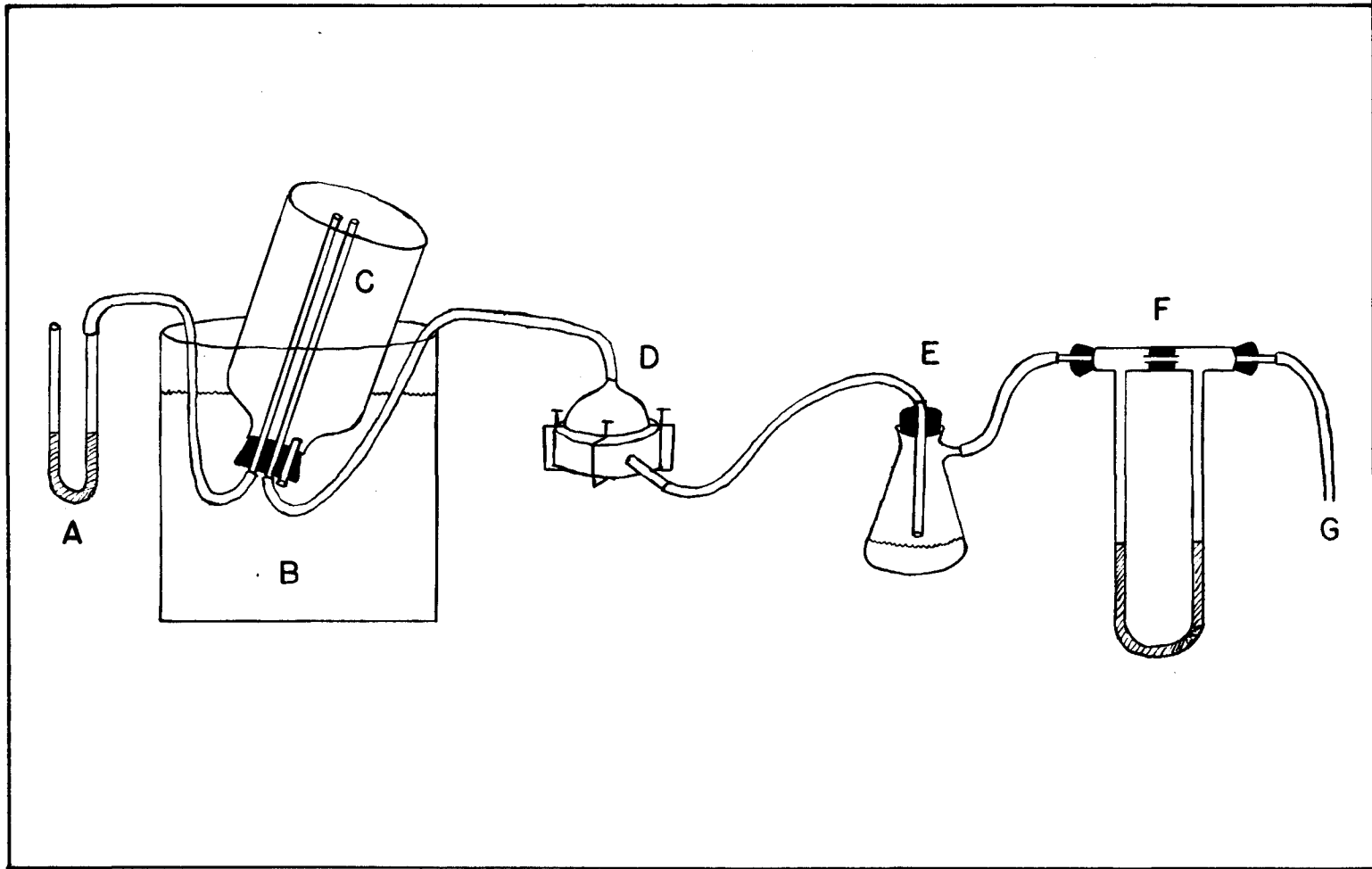
6. Atomizers

Figure 9 shows the various atomizers used. "A" is a coarse atomizer (De Vilbiss Company, Somerset, Pennsylvania) used only for spraying water to raise the relative humidity in the chamber. The rubber stopper on the atomizer was used to fit the device tightly into a hole in the side of the experimental chamber. "B" shows an atomizer specially made by the writer. It consists basically of a De Vilbiss atomizer with a glass tube 4 in long and 1 in in diameter attached in front of the nozzle. The short glass tube below and to the rear of the nozzle is an overflow reservoir for the nozzle tube. Since the atomizer was operated pointed upward at an angle of about 20° , liquid would flow through a small tube into the overflow reservoir. "C" is a De Vilbiss No. 44 nebulizer. All of the atomizers were operated at about 20 in mercury pressure.

7. Flowmeters

Air flowmeters used were of the type diagrammed in Figure 10, part F. However, a flowmeter such as described by Lemon and Wise (1944) was used occasionally, and was calibrated by means of a wet gas meter. The apparatus used

Figure 10. Apparatus used to calibrate air flow-rate. "A", water manometer; "B", tank of water; "C", 5-gal glass jar; "D", sieve air sampler with funnel clamped on top; "E", water trap; "F", flowmeter; and "G", line to vacuum pump



APPARATUS USED TO CALIBRATE AIR FLOW-RATE

to calibrate the former type of flowmeter is shown in Figure 10. For calibration, large tank "B" was nearly filled with water, and a 5-gal jar "C" having glass tubes in the rubber stopper as shown was placed inverted into the water. In the diagram a sieve air sampler is shown with an inverted funnel clamped on airtightly at "D". "E" is a small water trap used in the system when sieves were actually being used. "A" is a water manometer and line "G" leads to a vacuum pump.

Two persons were required to operate the calibration apparatus, one adjusting jar "C" in the water so that the air pressure in the jar would remain atmospheric as judged by manometer "A", and the other starting and keeping the air flow-rate constant (as judged by the mercury manometer on flowmeter "F") as well as timing the flow. When air was withdrawn from jar "C", water from tank "B" entered jar "C". Air flow was stopped before jar "C" was full of water. The tubes to jar "C" were then clamped or stoppered, the jar removed, and the volume of water in the jar measured. It was assumed that the volume of water in the jar corresponded to the volume of air which had passed through flowmeter "F" during the timed interval.

Each device requiring measurement of air flow was calibrated in a similar manner, i.e., all the units necessary in the line as it would be used in an experiment were

connected. The volume-measuring units "A", "B" and "C" of Figure 10 were then merely attached to the system at the end farthest from the flowmeter.

8. Timing of samples

In order that air samples might be timed with accuracy and convenience, a piston-operated valve such as used by May (1945, p. 190) was constructed and used for all air samples. The valve is so constructed that air flow-rate may be adjusted before sampling. At the moment desired for sampling to start, the piston is pushed connecting the vacuum line to the sampling device; to stop sampling, the piston is released. In Figure 2, the valve, with two lines attached, may be seen hanging near the left side of the table.

B. Mold Spores: Culture and Suspensions

1. Culture medium

The culture medium used was that of Moyer and Coghill (1946). These investigators developed the medium to be especially effective for sporulation of the genus Penicillium.

The pH of both agar and broth media was adjusted to 6.5 before autoclaving at 15 psig for 15 min.

To grow spores for aqueous suspension, 40 ml of broth were put into 6-oz prescription bottles and inoculated with spores from an agar slant culture. After the bottles were incubated on their sides for 3 to 4 days at temperatures of 25 to 30°C, a mycelial mat covered with spores was obtained. When dry spores were tested, spores from 2-per cent agar slants were used by blowing the spores out with air.

2. Mold culture, and preparation of mold suspensions

For all biological tests of germicides, Penicillium notatum spores were used, either dry or in aqueous suspension. Stock cultures were maintained as follows. A 4-day-old agar slant was placed in a cardboard tube and refrigerated for a 1-year period. At the end of a year a new working culture was taken from the stored tube and also another culture from the same tube was grown for refrigeration-storage for another 1-year period. Meanwhile the cultures for daily use were taken from sub-cultures transferred once a month and kept in a 25°C incubator.

Aqueous suspensions of spores were prepared by pouring off the broth from under a mycelial mat, adding about twenty-

five, 5-mm glass beads and about 50 ml of sterile distilled water, and shaking gently in a 1-ft arc 100 times. Then, as described by Mellody and Bigg (1946, p. 46), the spores were separated from the mycelia by filtration through three thicknesses of sterile cheesecloth.

In order that an appropriate number of spores might be atomized into the chamber for each experiment, the thickness of the suspension was first adjusted by eye to approximately the same concentration each time, and then the number of spores per unit volume was determined by actual hemocytometer count and in certain instances by plate counts. After an appropriate number of spores had been determined for atomization purposes this number could be fairly well repeated, no matter what the hemocytometer count, by computation using the formula

$$\text{volume} \times \text{concentration} = \text{volume} \times \text{concentration}.$$

3. Spore suspension characteristics

The age of spore cultures used in all studies discussed herein was 7 days or less, with the average being about 5 days. Examination of several spore suspensions of P. notatum were made under the microscope. The average diameter of

single spores was obtained from measurements of over 150 spores. From counts of over 1600 spores the percentage of cells existing singly or in groups of two, three, four, five, six and greater-than-six was obtained. The percentage of mycelial fragments was also determined.

From settling rates (open plate collection) and disappearance rates (sieve samples) of spores atomized (sprayer B, Figure 9) into a gently-fanned atmosphere, the average sizes of the air-borne particles were determined using Stokes Law (cf. Lidwell, 1948b, pp. 320, 321). According to Stokes Law,

$$s = 0.36 d^2 = Kgh$$

where d is the diameter in μ of a sphere of unit density (*P. notatum* spores were assumed to have unit density and the density of air was neglected), s is the settling rate in feet per hour, h is the height (in feet) of the enclosed space, and K_g is a "die-away" constant with units hours⁻¹ (to be discussed). Since h was known and K_g was determined experimentally, the average diameter of the spores could be calculated. This calculation was made.

The percentage of solids in the suspending liquid was determined subsequent to centrifugation of a distilled water suspension of the spores. Then five, 50-ml portions of the

centrifugate were transferred to previously dried and tared drying dishes. An 80°C oven was used to evaporate most of the liquid and then a vacuum oven at 100°C was employed to complete the drying.

C. TEG Determination

The TEG used in this study was Air Treatment Grade made by Carbide and Carbon Chemicals Company, New York. Diethylene and tetraethylene glycols were listed as 2 per cent by weight. It is recommended on the label that the temperature of the TEG in vaporizing devices be kept below 250°F to avoid thermal decomposition and possible formation of obnoxious fumes. Refractive indices of this TEG agreed closely with those of vacuum-distilled (with a short refluxing column) technical grade TEG of Eastman Organic Chemicals, Rochester, New York.

Determinations of aerial TEG concentrations were made by the method of Kaye and Adams (1950, pp. 661-663), a colorimetric determination. The air-sampling device used was an air washer, i.e., an air sample was bubbled through concentrated sulfuric acid in this instance.

Standard curves were obtained by pipetting known amounts of TEG and water into test samples. Since water taken in with air samples reduces the intensity of the color obtained,

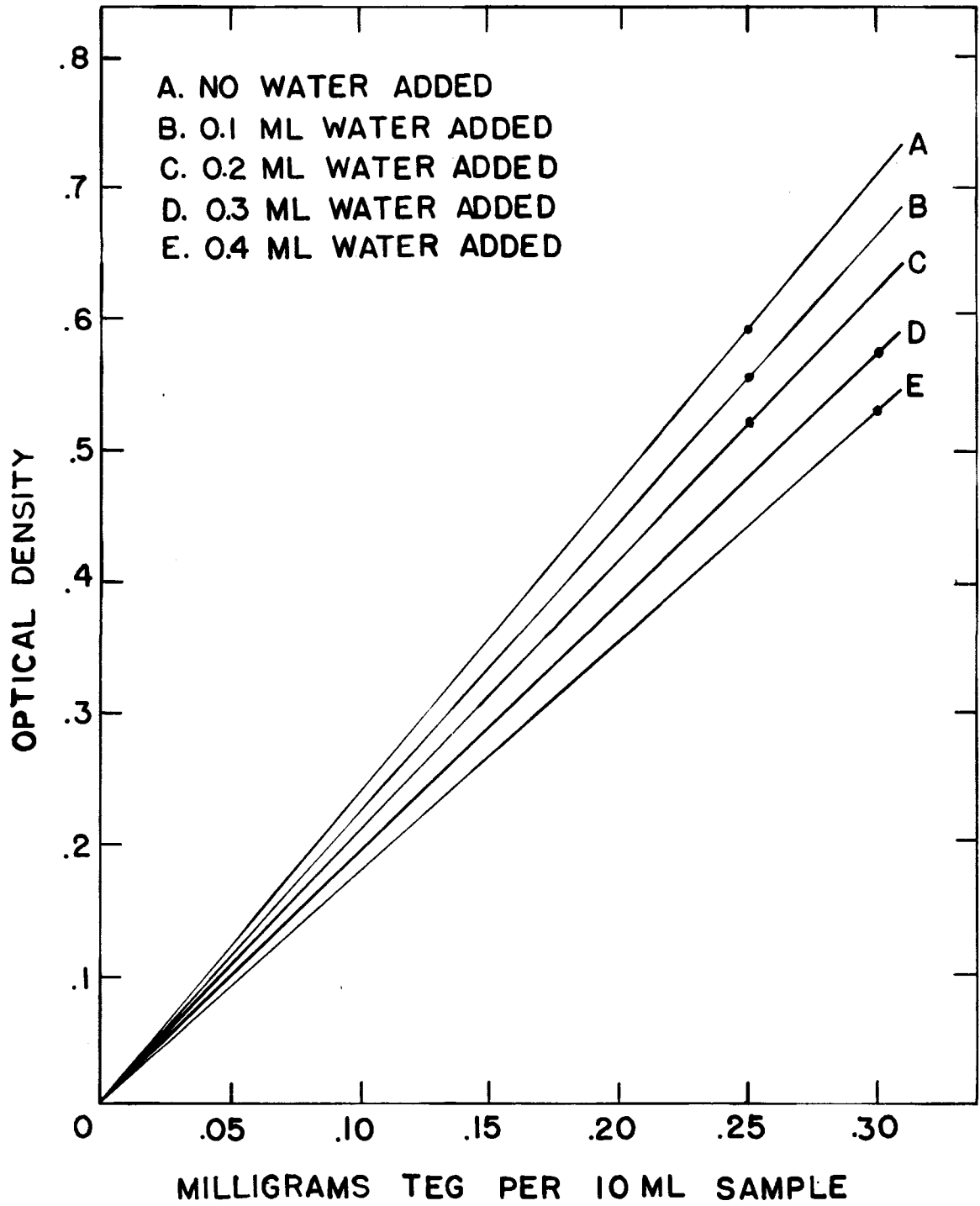
five curves (A through E, Figure 11) each with a constant amount of added water were determined. Kaye and Adams describe the relationship between optical density (transmittancy) and TEG concentration as being linear. Several experiments were run to verify this, and therefore in determining the straight lines of Figure 11 it was necessary to determine only one point in order to fix a line, each line passing through the origin.

Concentration is proportional to optical density in the color reaction used. This relationship is expressed by the equation

$$C = K (O.D.),$$

where C is the concentration of TEG in milligrams per 10-ml sample, K is a proportionality constant which is constant only when the amount of water in a sample remains constant (K is equivalent to the reciprocal of the slope of the line determined by any particular amount of water when optical density is plotted versus milligrams of TEG in a 10-ml sample (Figure 11)), and O.D. is the optical density. In order to determine the fluctuating constant K for different amounts of water in aerial samples, a plot was made of the logarithms of the reciprocal of the slopes versus the milliliters of

Figure 11. Standard curves obtained for determination of aerial TEG concentrations at various concentrations of water by the method of Kaye and Adams (1950, pp. 661-663)



water in a 10-ml sample (Figure 12). Aerial TEG concentrations were computed as follows:

$$\frac{\text{grains H}_2\text{O}}{\text{lb dry air}} \times \text{in Hg} \times \frac{1}{460 + ^\circ\text{F dry bulb}} \times \frac{\text{L air}}{\text{sample}} \times 0.00302$$

$$= \text{g H}_2\text{O in total collection fluid.}$$

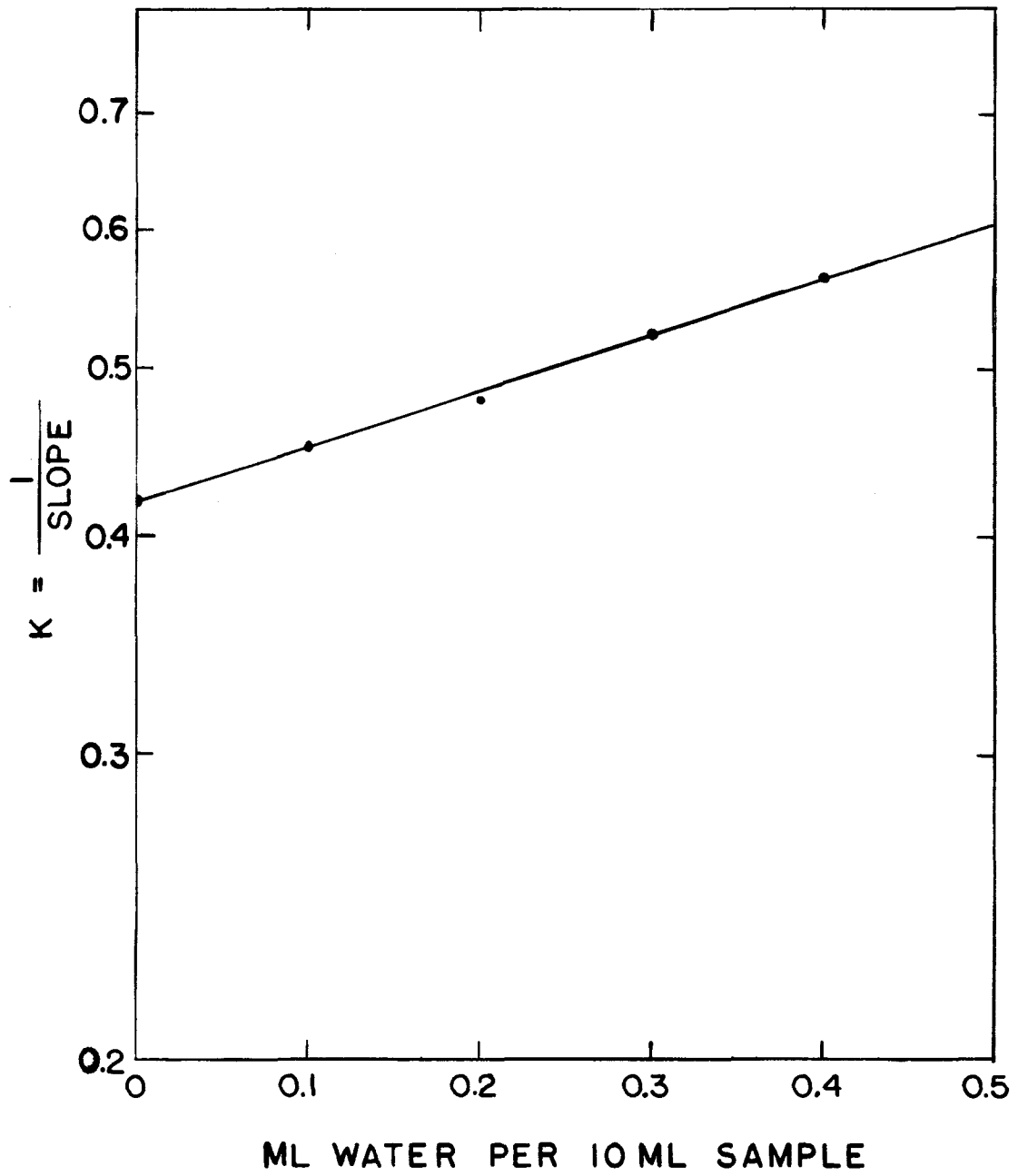
The constant 0.00302 represents the products and quotients of various conversion factors. The first term of the equation was obtained from a psychrometric chart, by using dry- and wet-bulb thermometer readings. By referring the computed answer above, adjusted to obtain grams water in a 10-ml sample of liquid, to the graph in Figure 12 the constant, K, applicable to the particular sample was obtained. This constant along with the optical density of the sample allowed computation of $\% \text{ TEG}$ for 1 of air. Aerial TEG concentrations will be expressed in these units.

D. Experiments with Possible Decomposition

Products of TEG

Glycolaldehyde, glyoxal, glyoxylic acid and glycolic acid were tested for their air-disinfecting properties against P. notatum spores. Glycolic acid, about 85 per cent

Figure 12. Relationship between the amount of water in a 10-ml, TEG-sulfuric acid sample and the reciprocal of the slopes, K , of the lines showing the relationship between optical density and milligrams TEG in the 10-ml sample (cf. Figure 11)



aqueous solution, lot no. 3217, was obtained from K and K Laboratories, Long Island City, New York. Glycolaldehyde was obtained from Aldrich Chemical Company, Inc., Milwaukee, Wisconsin. Glyoxylic acid was obtained as the sodium salt from a batch prepared by Dr. D. Metzler, Iowa State College, Ames, Iowa. The salt was converted to the acid by addition of an equivalent amount of reagent CP sulfuric acid. Glyoxal (lot no. 752774) was obtained from Fisher Scientific Company, St. Louis, Missouri.

Tests for the existence of some of the above compounds in vapors generated by TEG vaporization (Glycoaire vaporizer) were made by bubbling the vapors from the experimental chamber through 2,4-dinitrophenylhydrazine (2,4-DNPH) dissolved in a 2N hydrochloric acid solution. Bubbling into 1 L of solution was at the rate of 14.2 L per min over a period of 8½ hr at a relative humidity of approximately 20 per cent and an air temperature of approximately 75°F. The concentration of TEG in the air at equilibrium was not measured.

Derivatives from possible TEG-decomposition products were also made in the following manners: (1) TEG fresh from the sealed bottle was poured directly into 2,4-DNPH solution, (2) filtered air was blown through TEG heated in a boiling water bath, the air being carried over into 2,4-DNPH solution,

and (3) the residual TEG (about $\frac{1}{2}$ of the liquid) from a vacuum distillation at 98 to 105°C, using an oil bath, and 60 to 120 μ mercury pressure was put into 2,4-DNPH solution. Replacement of the atmosphere in the distilling apparatus was with nitrogen gas.

Some of the precipitates in the 2,4-DNPH solutions were compared with known compounds by paper chromatography and by absorption spectra measurements (Hammond, 1956) in a Beckman spectrophotometer, model DU. Solvents used in the chromatography were (1) n-heptane saturated with methyl alcohol, and (2) n-heptane, chloroform and nitromethane in the ratio 10:4.5:2, respectively. Paper chromatographs were sprayed with a 10 per cent aqueous potassium-hydroxide solution.

E. Typical Procedures and Calculations of Experiments

1. A typical air-disinfection experiment

The walls of the experimental chamber, along with surfaces of all pieces of apparatus including tubes and cords used in the chamber, were wiped very carefully with water to remove as nearly as possible all condensed vapor from the preceding experiment. Compressed air filtered through cotton at a rate of about 1 cfm was then used to flush the 31-cu ft

chamber for a minimum of 2 hr before the experiment. The outlet for the air was at the floor level.

While the chamber was being flushed, about 31 petri dishes were prepared with Moyer-Coghill agar. Spores 3 to 7 days old were harvested and prepared as described above. The sieve air samplers were washed, dried briefly in a low temperature oven and then cooled. The sieve tops of the samplers were quickly flamed and put into a large covered beaker until the samplers were again assembled.

Figure 5 illustrates the usual arrangement of sieve samplers and settling plates on the floor of the chamber. Lids of petri dishes matching the lower halves inside the assembled sieve devices were placed on the floor of the chamber during an experiment.

Just before the mold spores were atomized, air in the chamber was cooled if excessively hot, and the relative humidity of the air was adjusted by spraying in sterile distilled water or flushing with air passed through calcium-chloride tubes. When very low relative humidity was desired, magnesium perchlorate was also placed in dishes within the chamber. If TEG vapor concentration was to be measured, a control vapor sample was taken at this time.

Relative humidities for an experiment were measured just before or shortly after the introduction of the mold

suspension, at the time of a vapor sampling, and at the finish of mold-spore sampling. To allow for pressure adjustment in the chamber during sampling, a tube with a cotton filter was attached to an opening in the chamber wall.

Air sampling for the control period (no disinfectant) was usually started 2 to 3 min after the spores were atomized into the chamber. Intervals between sieve samples were about 2 to 5 min, the duration of the samples ranging from 10 sec to $1\frac{1}{2}$ min over the entire experiment. Settling dish exposures ranged from $\frac{1}{2}$ min to about 10 min, depending on the number of viable spores expected to fall. An effort was made to obtain colony counts of 250 or less from sieve samples. The desired number of colonies on settling dishes was 100 or less.

After about four air samples had been taken (using both settling plates and sieve samplers) to determine the control settling and die-away rates, TEG vaporization was started. After 1 to 2 min, sampling was again started to determine the total die-away rate. If TEG-concentration measurements were to be made, it was necessary (since there was only one operator) to suspend air sampling for about 4 min while TEG samples and thermometer readings (for calculation of relative humidity) were taken. Thus, TEG samples were usually taken before atomizing the spores, about midway in the vapor-test period, and at the finish of the experiment. For many experiments, however, TEG-concentrations were not measured.

Collection plates were incubated $2\frac{1}{2}$ days at 25 to 35°C and colonies developing were enumerated using a Quebec colony counter.

Tests in which glycerol, glyoxal, glycolic acid, glycolaldehyde, and glyoxylic acid were vaporized were conducted in a similar manner. In these experiments, however, no vapor concentrations were determined.

In computing the results, the counts obtained were converted to spores settling per minute onto standard-sized petri dishes (about $3\frac{1}{4}$ -in diameter), and spores per cubic foot of air as collected by the sieve samplers. An approximate correction was applied to account for dilution of the air in the chamber when sieve samples were taken. The corrected counts were then plotted as logarithm of spores per cubic foot of air (or logarithm of spores settling per minute onto a petri dish) versus time in minutes (midway in the sampling interval). Straight lines to fit the experimentally-determined points were drawn. In many instances a regression line was computed. Computation of die-away constants from these plots is discussed below.

2. Determination of "die-away" constants

"Die-away" or "rate of disappearance" constants have been discussed by Bourdillon et al. (1948, p. 62). In the

present study three different die-away constants are used, (a) K_S , to express the die-away per hour during the control settling period of an experiment, (b) K_T , to express the total die-away per hour resulting from settling plus germicidal action, and (c) K_D (equivalent to $K_T - K_S$), to express the die-away per hour resulting from effect of the germicide alone. The subscripts S, T, and D designate settling die-away, total die-away (settling plus death), and death die-away, respectively. Computation of a die-away constant was as follows:

$$K_S = \frac{138 (\log_{10} N_1 - \log_{10} N_2)}{t_2 - t_1}$$

where N_1 and N_2 are counts of organisms in similar-sized samples at times t_1 and t_2 , respectively. Values of t are expressed in minutes, while K values are in hours.

F. Viability of TEG-Exposed Spores

An experiment designed to test the viability of air-borne *P. notatum* spores before and after exposure to TEG vapor, and to demonstrate the presence of non-viable spores in the air after their exposure to TEG vapor was conducted. Three ml of sterile Moyer-Coghill agar were spread onto each of

several sterile glass microscope slides. Each slide was placed into a petri dish so that the dish and slide might be placed inside a sieve sampler to be exposed to an air sample. An extremely heavy aerial suspension of spores was atomized (sprayer B, Figure 9) into the chamber, and TEG vaporization started. Sieve samples of 1 to 2 cu ft of air were taken at various intervals of time onto the agar slides. The same procedure was repeated again with the same mold suspension after the chamber had been cleaned and flushed, except that no TEG was used. The agar slides were incubated in a moist atmosphere for 14 hrs at 25°C after which coverslips were placed on the agar and the agar examined under the microscope. The percentage of spores germinating in each small group of spores which had been deposited beneath a sieve hole was calculated for the control and the vapor experiments.

The same type of experiment was repeated, except that the period of incubation for some agar-slides was lengthened to 5 days. In addition, photomicrographs were taken of some fields.

G. Miscellaneous Experiments

1. Experiments without settling plates

A few experiments made early in this study were conducted without settling plates. Air sampling was with the Moulton collector, and vaporization was by means of the steel plate described previously. In these experiments the chamber was filled with the vapor and the concentration measured only before spores were injected. Spores were allowed to mix in the germicidal atmosphere for 10 to 15 min after which a single air sample was taken and the percentage of survival calculated from results of a control experiment using the same spore suspension.

2. In vitro experiment using glycerol

P. notatum spores in a concentration of 100,000 per ml were tested in aqueous solutions of glycerol of 0, 10, 40, 80, and 98 per cent. Exposure was for 2 hrs, after which 0.1 ml of the suspension was diluted and plated in Moyer-Coghill agar, using three plates per glycerol concentration level. An analysis of variance was computed to detect any

differences among survivals in the various concentrations of glycerol.

3. Experiments at different temperatures

One test of the killing power of TEG vapor with respect to the same spore culture used as an aerosol at 83°F and 105°F was conducted. The effect of the lower temperature was measured first while the spores remained in aqueous suspension for 4 hr until the second test. To raise the temperature for the second test, a 1000 w heating coil was suspended from the top at one side of the chamber. The coil was used only prior to the start of the experiment.

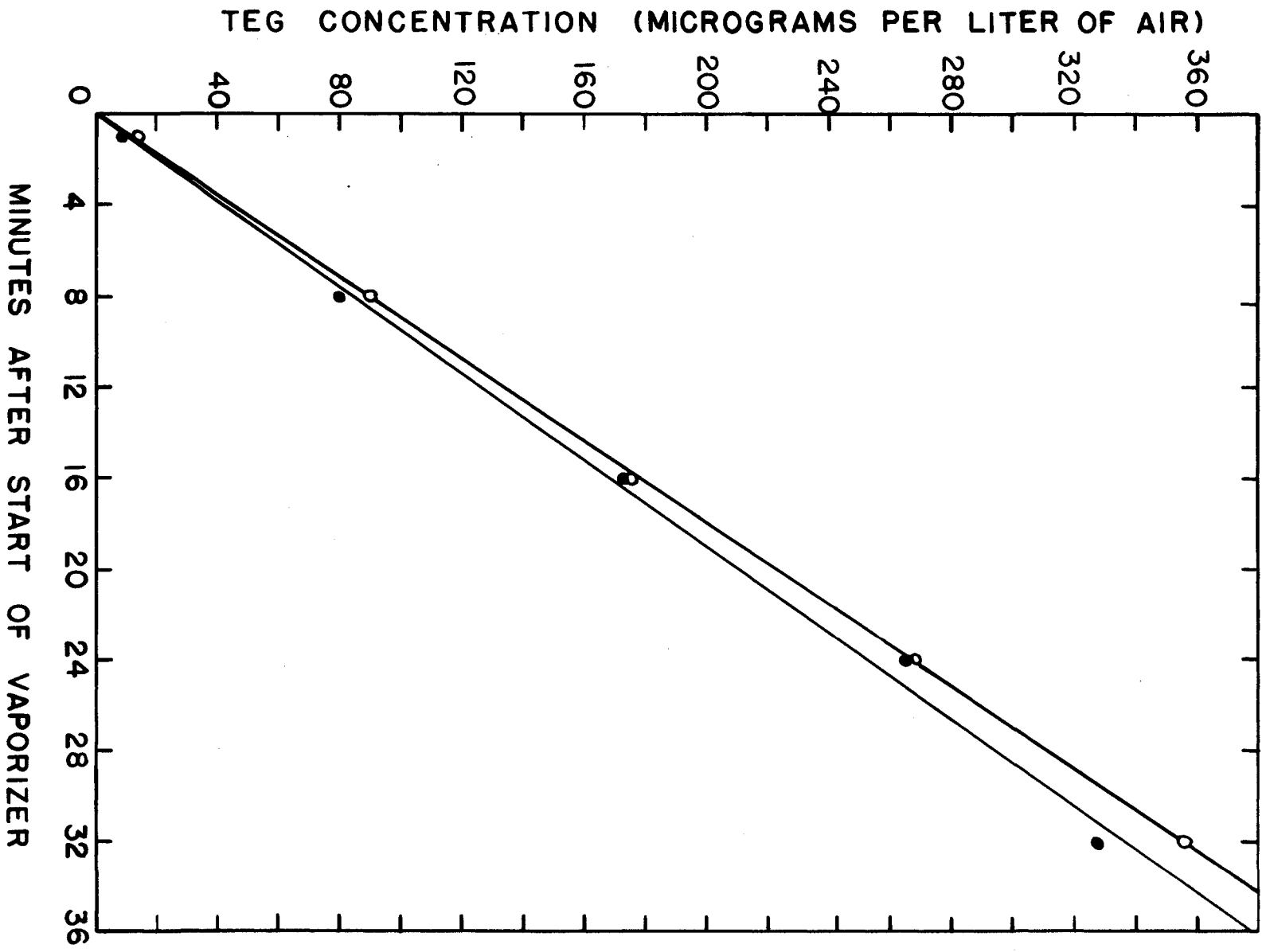
IV. RESULTS AND DISCUSSION

A. Performance of Equipment, and Properties
of Spore Aerosols1. Vaporizer output

Figure 13 shows graphically the increase of TEG concentration in the chamber with time when the Glycoaire vaporizer was run at full capacity (110 v). Measurements were made in the relative humidity range of 47 to 53 per cent and at temperatures of 76 to 84°F. The temperatures to which the TEG was momentarily exposed were never greater than 200°F except possibly when the glass tape was slightly wrinkled.

In studies of the fungicidal effect of TEG vapor, 15 per cent relative humidity was the lowest tested. At this relative humidity the greatest amount of TEG would be required to saturate the air. About 10 % of TEG are required to saturate 1 L of air at 15 per cent relative humidity and 86°F (Wise and Puck, 1947, p. 556). It may be seen, therefore, that the atmosphere in the experimental chamber was saturated in 1 min or less in all experiments where full capacity of the vaporizer was used.

Figure 13. Graph showing the increase in aerial TEG concentration in the experimental chamber during separate experiments when the commercial vaporizer (Glycoaire) was run at full capacity



2. Variability among sieve samples

Colony counts from ten sieve samples ranged from 193 to 311 per plate, with an average close to 250. When these counts were corrected for dilution of the chamber atmosphere and converted to spores per cubic foot of air the range was 1213 to 1866, with a mean of 1515 and a standard deviation of the counts adjusted to represent deviations from a common regression line of ± 95 , the variation being ± 6 per cent.

3. Reproducibility of air-volumes using flowmeters

Five trials were made to calibrate one flowmeter. The mean amount of air displaced in the jar was 5.689 L per 0.2 min and the standard deviation ± 0.2293 .

4. Mold spore characteristics

Forty spores of P. notatum in aqueous suspension were found to have an average diameter of 3.27μ . (These spores had been grown on broth for 7 days.) From measurements made on spores from two different agar-slant cultures (4 days old), average spore diameters of 3.18μ and 3.10μ were obtained from observations of 60 and 40 spores, respectively.

The distribution of spores, clumps of spores, or mycelial fragments from one spore suspension (5-day-old culture) was determined by counting the various particles (over 1600) in a hemocytometer. The results are summarized in Table 1.

Table 1. The distribution of spores, clumps of spores, and mycelial fragments in a suspension of spores of P. notatum

Class of particle	Percentage of total
1-cell	79.3
2-cell	10.9
3-cell	3.50
4-cell	2.00
5-cell	0.94
6-cell	0.31
greater than 6-cell	0.94
mycelial fragments	2.10

Since the amount of solid material in an aerosol droplet has been observed to influence the survival of organisms within the droplet when evaporated (Dunklin and Puck, 1948, pp. 87-101), fluid from another spore suspension (spores removed) was dried. The total solids content amounted to

0.1 per cent. This small amount of solid material when concentrated by evaporation of aerosol droplets might cause significant mortality of sensitive organisms, but it is thought that mold spores used were relatively resistant to the effects of desiccation.

Data from six experiments at relative humidities between 40 and 52 per cent (experiments 4 through 9, Table 2) were averaged and the die-away constant K_g was found to be 1.66. The average diameter of aerosol particles suspended in air at these humidities was then calculated (using Stokes Law) as 3.9μ . When data concerning settling particles were used from the same experiments (excepting experiment 6 where data were not obtained), the average diameter of these particles was calculated as 9.3μ . The diameters calculated above are equivalent to those of spherical water droplets of the same size, since in the use of Stokes Law the density of the spores was assumed to be unity and the shape of the particles spherical. P. notatum spores are very nearly spherical by microscopic observation, and the density of other microorganisms suspended in air at relative humidities of near 50 per cent has been found to be near to unity (cf. Orr and Gordon, 1956).

In order to verify the assumption that the normal die-away of the air-borne mold spores in the experimental chamber approximated linearity when logarithms of numbers were

Table 2. Data from experiments at different percentages relative humidity

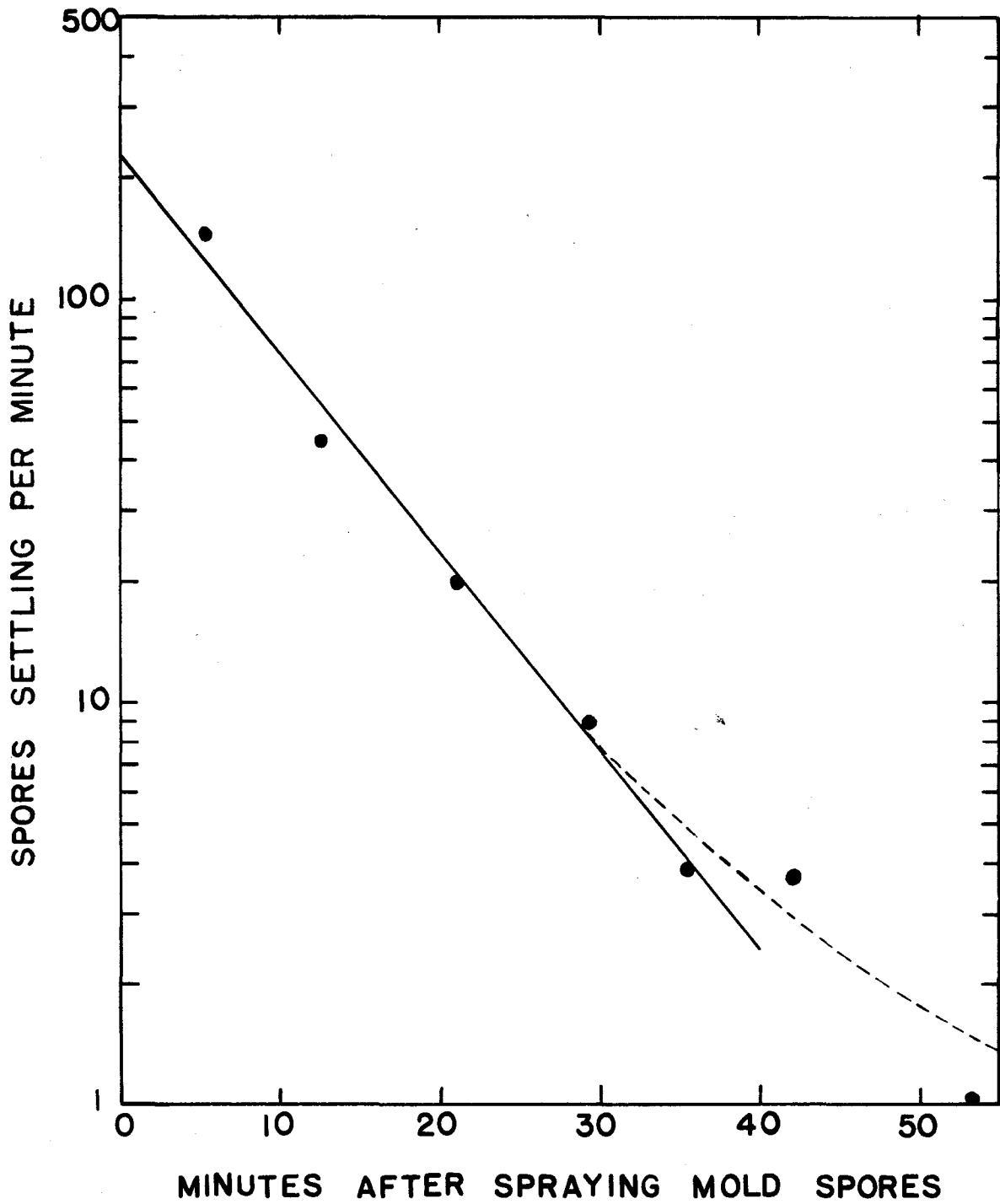
Expt. no.	Av. rel. humid.	Range of rel. humid.	Av. temp.	Range of temp.	Sieve data		Settling-plate data	
					K _S ^a	K _D	K _S	K _D
	(%)	(%)	(°F)	(°F)				
1	15.5	15.0-16.0	79.0	77.0-80.5	1.8	-0.05	7.4	0.29
2	21.5	20.5-22.5	81.0	79.0-82.5	2.1	5.1	5.5	1.9
3	31.0	29.5-32.0	78.0	76.5-79.0	1.2	12	12	3.8
4	41.5	43.0-40.0	85.0	84.5-85.0	1.4	25	8.9	18
5	42.0	41.0-43.0	83.0	79.0-86.0	2.1	20	9.2	13
6	45.0	49.0-41.0	77.0	75.0-79.0	1.5	14	---	11
7	46.5	52.5-40.5	75.5	73.0-78.0	1.7	18	8.8	8.1
8	49.0	51.0-47.0	82.5	81.6-83.0	0.60	20	10	19
9	50.0	52.0-47.5	80.5	80.6-80.3	2.7	12	11	3.8
10	53.0	59.0-47.0	78.0	76.0-80.3	2.3	16	11	11
11	54.0	59.5-48.0	78.0	75.0-79.5	2.4	13	10	10
12	64.0	71.0-57.5	84.0	82.6-85.3	5.7	17	17	7.4
13	74.5	83.0-66.0	79.0	77.3-80.6	5.7	1.4	12	-2.6
14	81.0	85.0-76.5	87.0	88.0-86.0	5.8	3.9	10	-0.59
15	81.0	80.0-83.5	87.0	85.0-88.5	3.0	3.7	8.7	-1.4
16	90.0	94.0-86.0	85.0	85.5-84.0	8.1	-1.3	12	-4.1

^aAll K values are expressed as die-away per hour. K_S = die-away constant for control period. K_D = die-away constant for fungicidal action alone.

plotted versus time, an experiment was conducted similar to those used in all control periods of other studies, except that the period of observation was extended to about 55 min. Gentle mixing of the atmosphere with a fan was used. The relative humidity remained at 50 to 52 per cent, while the temperature remained very close to 76.5°F. Collection was by settling plates.

In Figure 14 the results of the above experiment are shown. It may be seen that a good approximation to linearity held for the first 30 min die-away of spores, while a decrease in settling rate appears to have taken place gradually, presumably because of the removal of the larger, more-rapidly-settling particles from the air. Therefore, in all experiments where an assumption of linearity was necessary, data beyond 30 min were not used. Had the spore aerosol been homogeneous with respect to particle size, one would have expected the settling rate to remain constant throughout settling (i.e., in equal time intervals, a certain constant percentage of the remaining spores would have settled). This would be a geometrical decrease in the number of spores deposited during equal time intervals. However, when dealing with material of graded sizes (such as the spore suspension used herein) one might expect the mean rate of settling to decrease with the more rapid removal of the larger particles.

Figure 14. Graph of the settling of spores as collected by settling plates. The relative humidity was 51 per cent and the temperature 76.5°F. Decrease in settling rate is indicated by the broken line



Nevertheless, it appears that the rate of settling remains fairly constant in the present case (Figure 14) for the first 30 min. Further data as to the settling behavior of the spore suspensions used are presented below.

A second settling experiment was made at 48 per cent relative humidity and 76°F, in which no fan was used except during the atomization of the spores. Since Phelps (1942, pp. 134, 135) concluded that inevitable minor convection currents within a chamber were sufficient to maintain a substantially-uniform distribution of particles, it is thought that in the present study the thermal currents and the moving of petri-dish lids in the chamber were sufficient to keep the spore particles uniformly distributed. Results of this experiment are presented in Figures 15, 16 and 17. Figure 15 shows the rapid settling of spores in the first 30 min (rectangular coordinates). Figure 16 exhibits the same data plotted on semilogarithmic coordinates. It may be seen that an approximation to linearity might be made during the first 26 min. Figure 17 is a plot of the same data, except that logarithmic coordinates were used. In this graph a change to linearity is seen at 26 min. The results presented in Figures 15, 16 and 17 serve to describe the nature of the mold aerosol used throughout the present studies and were therefore included here, but the settling experiment presented

Figure 15. Graph showing settling of P. notatum spores in relatively quiet air. The relative humidity was 48 per cent and the temperature 76°F

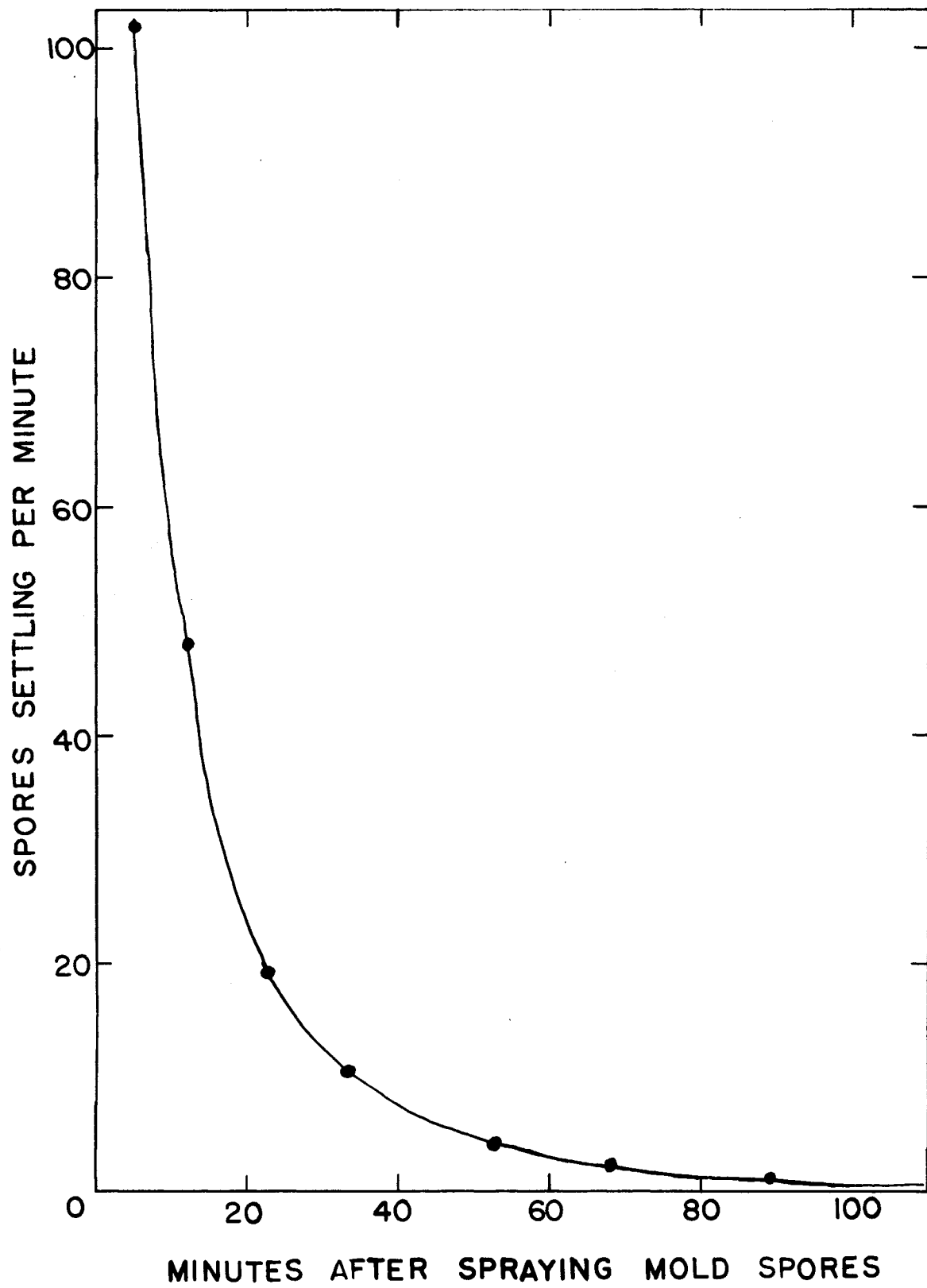


Figure 16. Graph showing data from Figure 15, except that the plot has now been transferred to semi-logarithmic coordinates

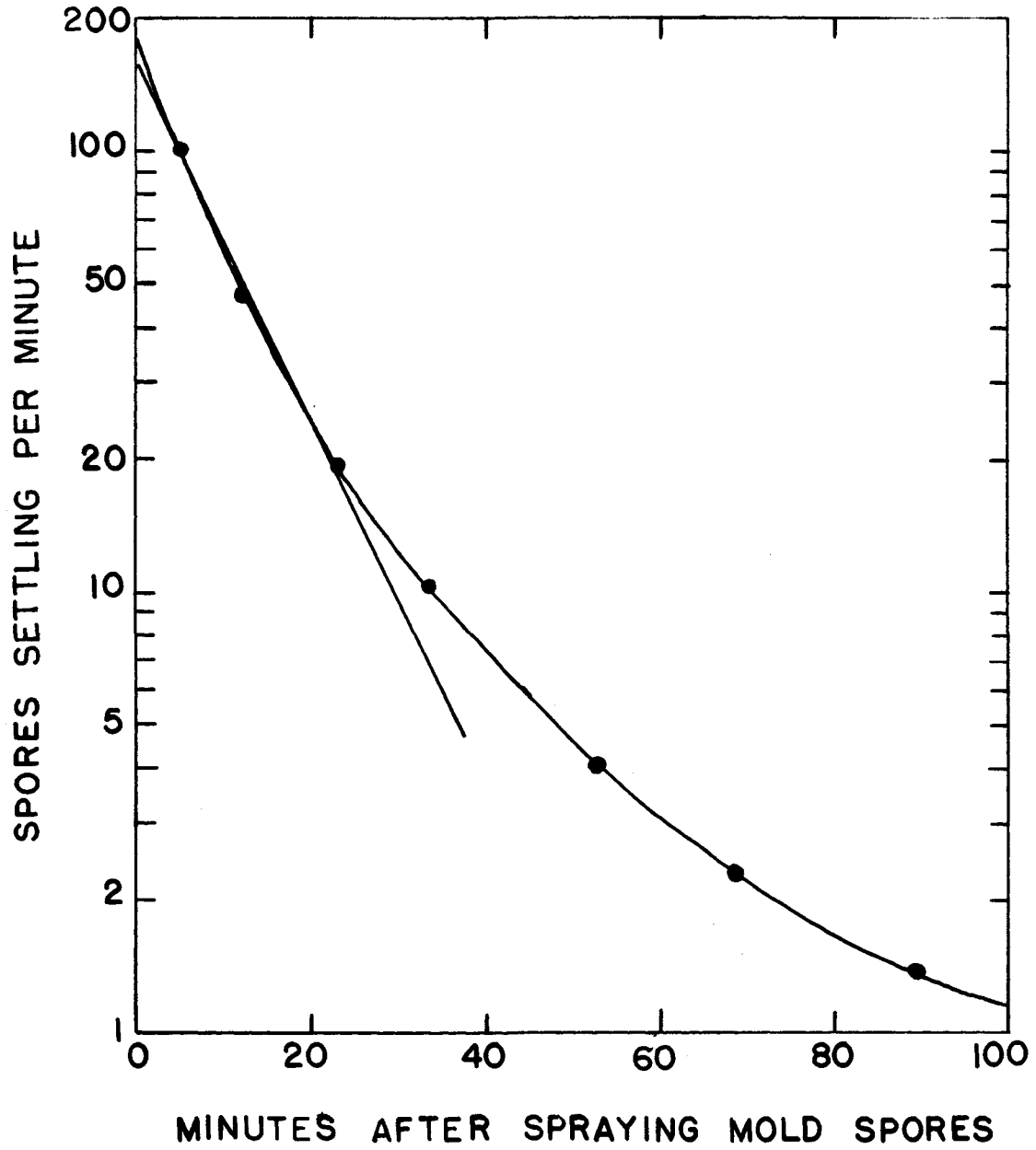
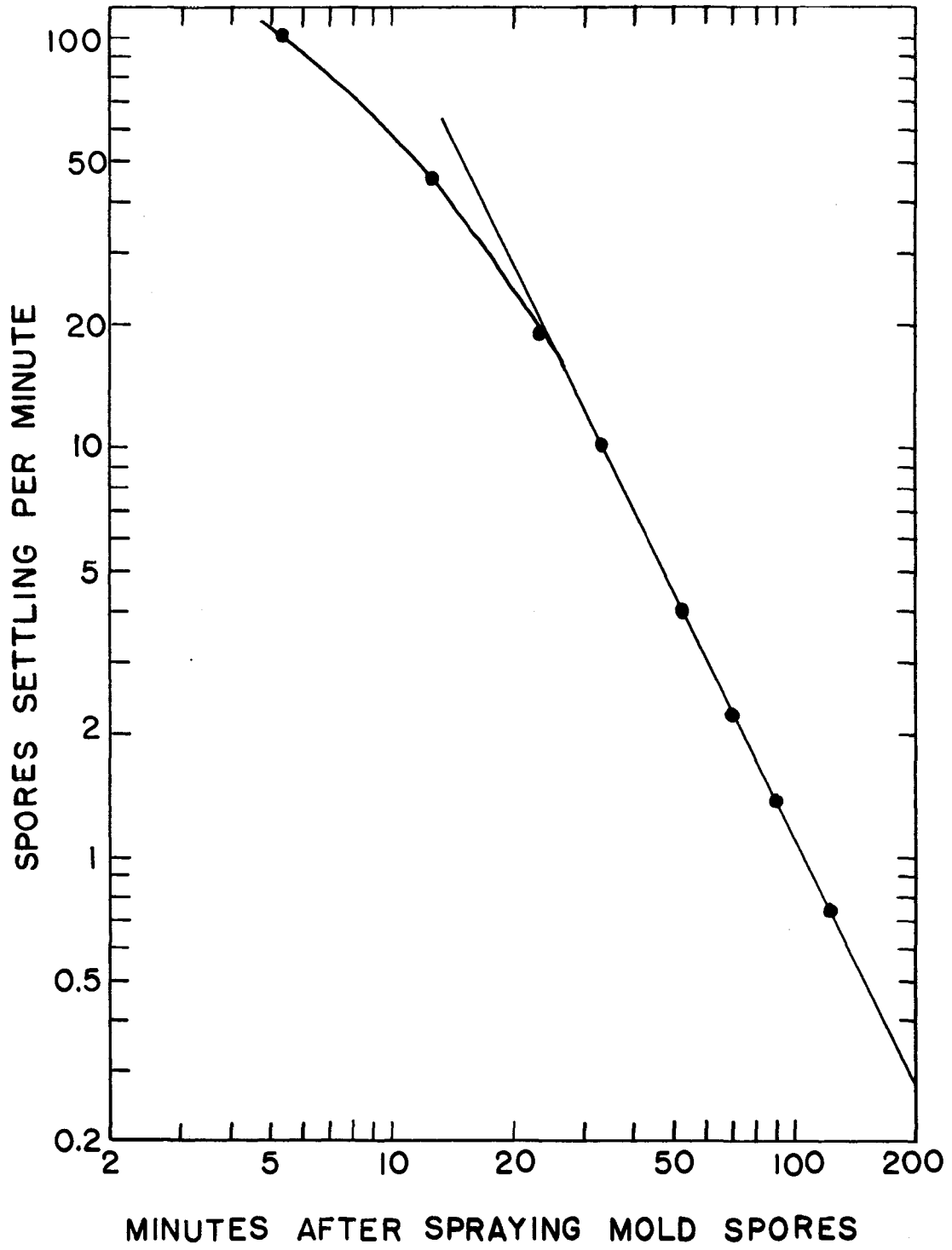


Figure 17. Graph of data from Figure 15, except that the plot has now been transferred to logarithmic coordinates



in Figure 14 represents actual conditions (use of fan) under which the data were gathered.

B. Effect of Vapor Concentration

Table 3 gives data from five experiments made at an average relative humidity of 49 per cent and in a temperature range of 75.0 to 82.5°F. Relative percentages of saturation of the air with TEG vapor have been interpolated from a graph given by Wise and Puck (1947, p. 556). The percentages tested in the present study ranged from 57 to 96. Significant killing of smaller air-borne spore particles (collected by sieve) was observed to develop between 67 and 78 per cent of saturation. A concomitant but less effective increase in the rate of killing was observed in the case of larger spore particles (collected by settling plate).

Data from experiments at different relative humidities are presented in Table 2. All of the experiments, with the exception of number 8, were conducted in atmospheres at least moderately supersaturated with TEG. Concentrations were not measured, but in all cases a definite fog was visible without the aid of a strong beam of light. In order to prevent marked supersaturation in several of these experiments, the current to the vaporizer heater was reduced after vaporization

Table 3. Data from TEG-concentration experiments

Expt. no.	Av. rel. humid.	Range of rel. humid.	Av. temp.	Range of temp.	Av. TEG conc.	Satn.	Sieve data		Settling-plate data	
							K_S^a	K_D	K_S	K_D
	(%)	(%)	(°F)	(°F)	(γ/l)	(%)				
1	49.0	52.0-45.5	75.0	75.0-75.3	1.82	57	0.25	2.2	8.7	-1.4
2	49.5	53.0-46.0	79.0	78.5-79.5	2.46	63	1.1	2.4	8.4	-1.7
3	49.0	53.5-45.0	79.0	77.6-80.0	2.27	67	0.97	5.5	10	-2.1
4	49.0	51.0-47.0	82.5	81.6-83.0	3.75	78	0.60	20	10	19
5	49.0	52.5-45.0	79.0	78.3-80.0	3.70	96	1.9	17	9.4	10

^aAll K values are expressed as die-away per hour. K_S = die-away constant for control period. K_D = die-away constant for fungicidal action alone.

proceeded a minute or so at full capacity. In scanning the K_D (die-away) values in column 7, Table 2, only one (experiment 4) is found to be larger than that of the K_D determined for an atmosphere of 78 per cent saturation (experiment 4, Table 3). This suggests that an atmosphere supersaturated with TEG is no more effective in killing P. notatum spores than an atmosphere which approaches saturation.

Figure 18 presents a graph of the K_D values from five experiments (listed in Table 3) made with subsaturated TEG atmospheres as well as K_D values from three experiments made with supersaturated TEG atmospheres. The range of average relative humidities for these experiments was close (47 to 53 per cent). It may be observed from these data that at optimal percentages of relative humidity the K_D value for the smaller particles (sieve collection) appears to reach an average of approximately 16 (the equivalent of 16 air changes per hour). The curve indicates rapid rise in the fungicidal effect of TEG vapor observed between 2/3 and 3/4 saturation.

C. Effect of Temperature

Results of a brief temperature study in which the same spore suspension was exposed to TEG vapor at two different temperatures are shown in Table 4. The average relative

Figure 18. Broken-line graph showing the approximate relationship between aerial TEG concentration and die-away rate, K_D , of P. notatum spores. The three points at the right of the figure represent results from supersaturated atmospheres. Sieve samplers were used

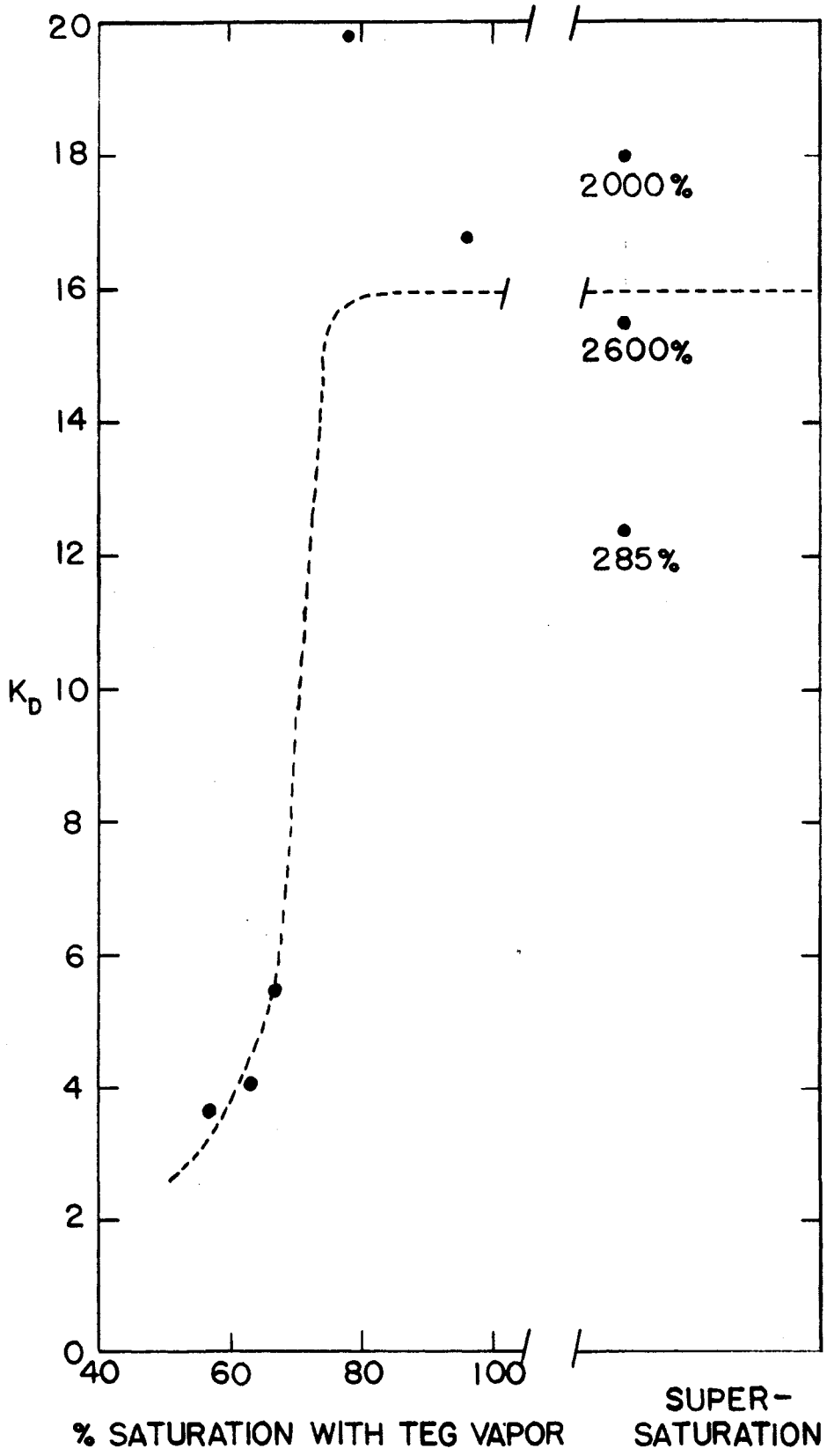


Table 4. The effect of temperature on the die-away of spores of P. notatum exposed to TEG vapor

Average temperature (°F)	Sieve data		Settling plate data	
	K_S^a	K_D	K_S	K_D
83	2.1	20	9.2	13
105	2.4	about 45	12	about 47

^aAll K values are expressed in hours⁻¹.

humidity during each test was 42 per cent (range 41 to 43). During the 83°F experiment the temperature rose from 79 to 86°F, while during the 105°F experiment the temperature fell from 106 to 104°F. Because of the unexpectedly rapid killing at 105°F, only two points were determined for each vapor curve, and thus the K_D values are approximations. Although a definite increase in killing rate with both small and large spore particles was evidenced at the higher temperature, a portion of this increase may have resulted from increased vulnerability of the spores because of incipient germination (4 hr in distilled water) prior to atomization. It is doubtful that a coefficient calculated for this range of temperature (83 to 105°F) would hold for the range of temperatures (77 to 85°F) at which the rest of the experimental data were collected. Jordan and Jacobs (1946a) calculated and compared four temperature coefficients (based on two

different formulas) from the same data collected using E. coli versus liquid phenol. They found that none of the coefficients remained constant over equal segments of temperature rise. A new and more constant (but involved) temperature coefficient was developed by these workers (Jordan and Jacobs, 1946b). Data collected in the present experiment were too meager to attempt to calculate a temperature coefficient for the fungicidal reaction tested.

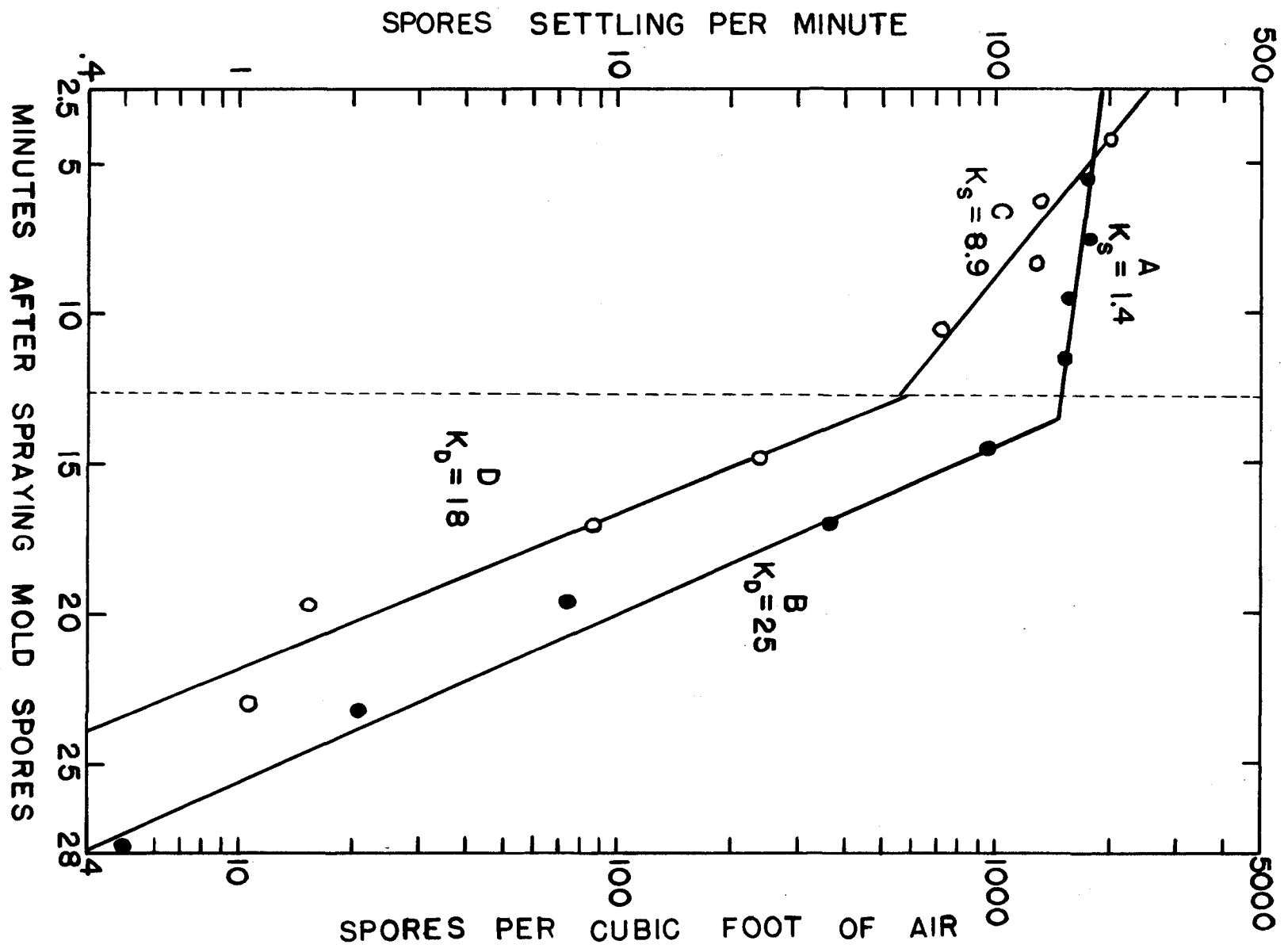
Data on the effect of temperature change on the fungicidal action of TEG vapor in the range 77 to 85°F would have been useful, but this information was not collected. Although there undoubtedly would be some effect upon the rate of action of TEG vapor if the temperature of the atmosphere were raised from 77 to 85°F, this effect was neglected when comparing experiments conducted within this temperature range. It was found that with the equipment available, chamber temperatures could not be controlled precisely. While the cooling system would reduce chamber temperatures prior to the start of an experiment, the cooling system was not used during an experiment, since TEG vapor and mold spores would have been trapped in the cooling ducts and on the refrigerated coils. On hot days, chamber temperatures would rise rapidly despite the insulation on the chamber. Moreover, a constant cause for temperature rise was heat from the vaporizer.

Although the effects of an 8°F temperature differential in comparing tests showing effective fungicidal action were not ascertained, the data presented in the present study are assumed to closely approximate the facts. Note that in spite of the use of relatively high temperatures in several experiments, the fungicidal effects were insignificant at the higher relative humidity levels (Table 2). In order to smooth any irregularities caused by temperature differences, the K_D values of two or three experiments at similar relative humidities have been averaged and plotted as a single result (cf. histograms discussed in the following section).

D. Effect of Relative Humidity

Results of a typical test for the fungicidal action of TEG vapor on air-borne mold spores are given in Figure 19. The percentage relative humidity used (41.5 per cent) was in the effective range; the average temperature was 85°F. Lines A and B represent data from sieve samples (use ordinate to the right), while lines C and D are from settling-plate data (use ordinate to the left). The left ordinate designates spores per minute settling upon the area of a standard petri dish. In the control portion of the experiment it may be seen that the mean die-away of the large particles ($K_g = 8.9$)

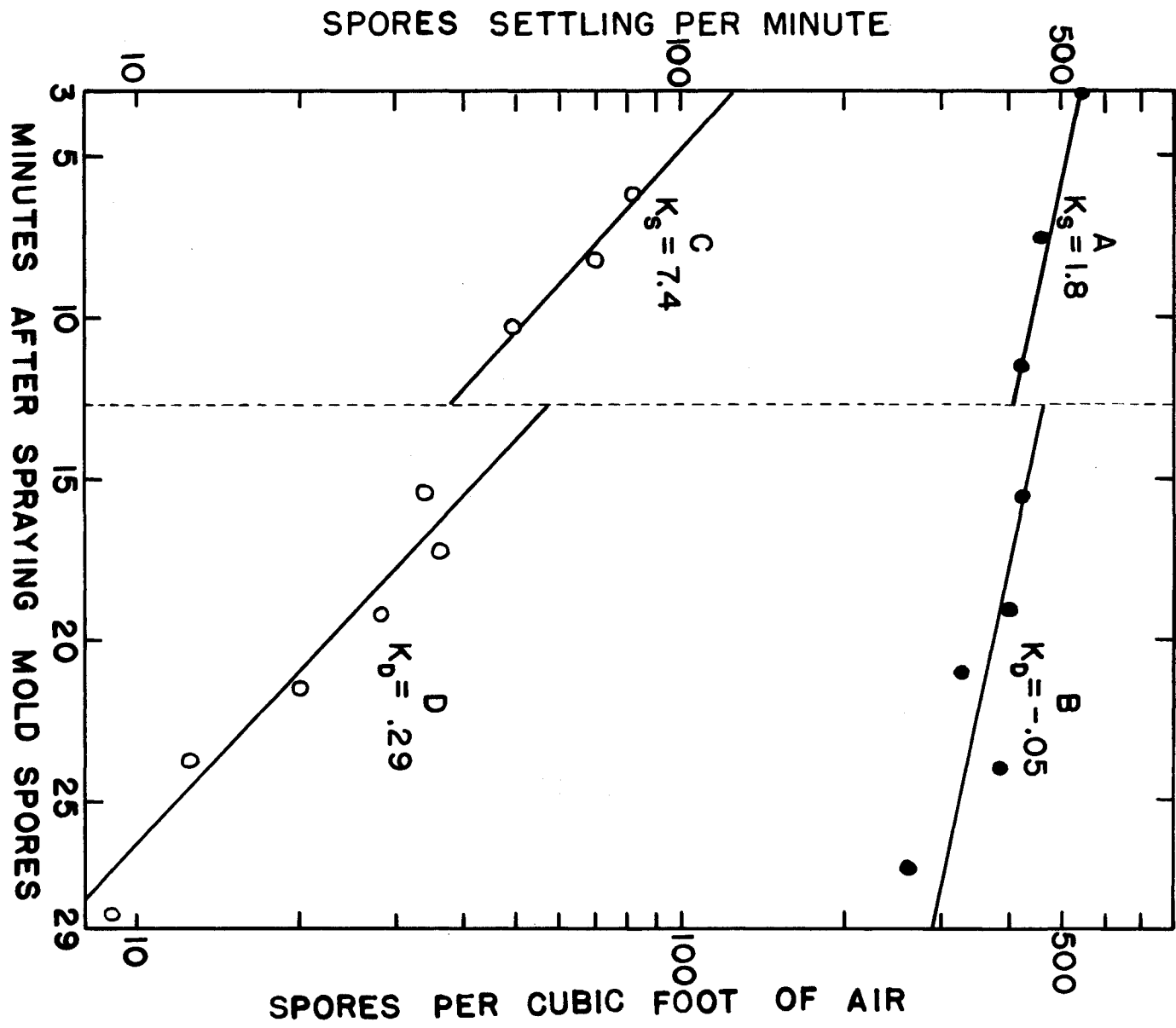
Figure 19. Graph showing results of a typical test for the fungicidal action of TEG vapor on air-borne P. notatum spores. The dotted line represents the point at which TEG vaporization was started. Lines A and B represent data from sieve collection, while lines C and D are from settling-plate data. Die-away constants are placed beside the lines from which they were derived. The relative humidity and temperature of the experiment were 41.5 per cent and 85°F



which settle rapidly on open dishes is much faster than that of the smaller particles ($K_S = 1.4$) which remain suspended in the air to be collected by sieve samples. Upon introduction of TEG vapor (moderately supersaturated) the smaller particles were more sensitive to the killing action of the vapor, i.e., K_D for sieve samples was 25, while for settling-plate samples K_D was 18. These values represent removal (above that resulting from normal settling and any other causes) of viable spore particles from the air at rates equivalent to 25, and 18 complete air-changes of the chamber per hour, respectively. The fraction of the original number of particles remaining after "x" air-changes of an enclosed space is given by e^{-x} (Bourdillon and Lidwell, 1948c), and thus the fraction remaining after 25 air-changes in the above instance would be $1/(2.718)^{25}$.

Figure 20 illustrates the complete lack of fungicidal power of TEG vapor observed when the relative humidity was 15.5 per cent and the temperature 79°F. The significance of the symbols and other markings on the graph are the same as on Figure 19. It is thought that the upward shift of line B at the point where the TEG vapor was introduced (dotted line) might possibly be caused by an increased efficiency of the sieve sampling device for larger particles formed by condensation of TEG vapor upon the spores.

Figure 20. Graph showing the ineffectiveness of TEG vapor against P. notatum spores at 15.5 per cent relative humidity and 79°F. Lines A and B represent data from sieve collection, while lines C and D are from settling plate data



It is also evident that line D does not form a smooth junction with line C. The increased colony counts above those expected from normal die-away were not an uncommon occurrence with settling-plate data from experiments conducted near the fringe of effective relative humidities. Sometimes the counts would increase and then fall somewhat more rapidly than would be expected from normal settling. At other times the settling-plate counts after vapor introduction at high relative humidities would continue to become higher than expected during the period of sampling (e.g., Figure 22, line D). It is the opinion of the writer that the explanation for this behavior may lie in the condensation of TEG vapor onto the spore particles thus increasing their size and weight, and yet not producing a killing effect because of inability to diffuse rapidly into the casehardened particles at low relative humidities or because of excessive dilution with condensed water vapor in the case of high relative humidities.

As evidence in support of the above hypothesis glycerol was vaporized in place of TEG in one experiment. (Glycerol had been found to be a very weak disinfectant against spores of P. notatum in vitro, e.g., after 2 hr of exposure a control sample had a count of 116,000 spores per ml, while counts from glycerol concentrations of 80 and 98 per cent

were 110,000 and 81,000, respectively.) Figure 21 shows the results of the experiment using glycerol. There was very little if any killing of small particles sampled with sieves. The effect on larger particles (settling plate data) was a gradual increase of counts above the values expected from continuation of the control settling curve. A decrease in slope resulted then, when the killing apparently was inconsequential, and when there was a presumed increase in settling of particles because of condensation of glycerol upon them. If the test had been continued, one would have expected the settling plate line to increase in slope, so that the overall settling period would have been shortened because of the faster settling.

Using the above hypothesis, the behavior of settling plate counts, which increase and then fall somewhat more rapidly than would be expected from normal settling, might be explained by delayed diffusion into the particle. Or, one might reason that since there were more particles settling immediately upon vapor introduction, there would be fewer left to settle, and the overall effect would be to produce a die-away line with a steeper slope. In the case where settling-plate counts after start of TEG vaporization were greater than and continued to be higher than expected (e.g., Figure 22, line D), one might reason that the killing

Figure 21. Graph of a test of the effect of glycerol vapor against air-borne spores of P. notatum. Symbols have the same significance as in Figure 19. K_T = total die-away resulting from all causes

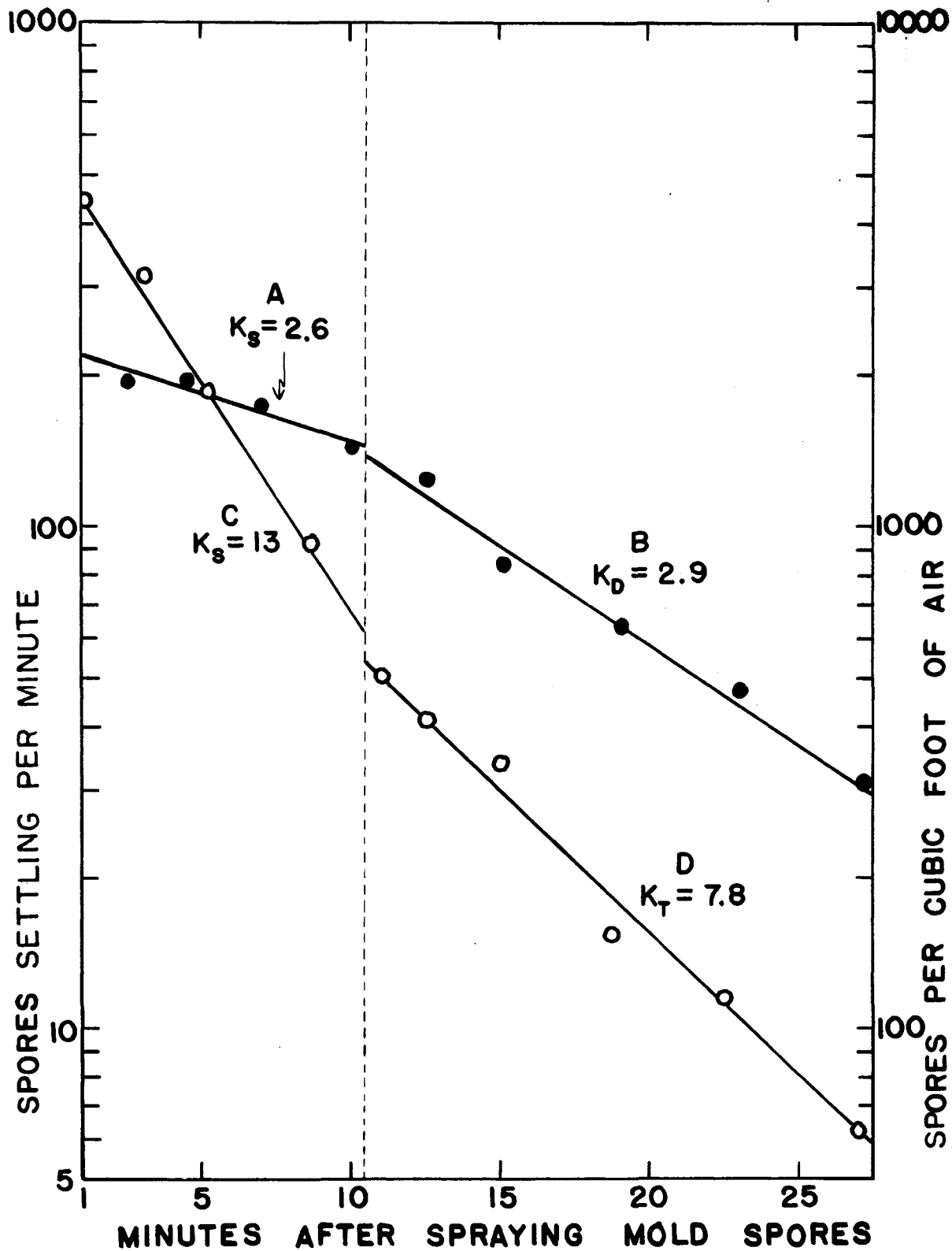
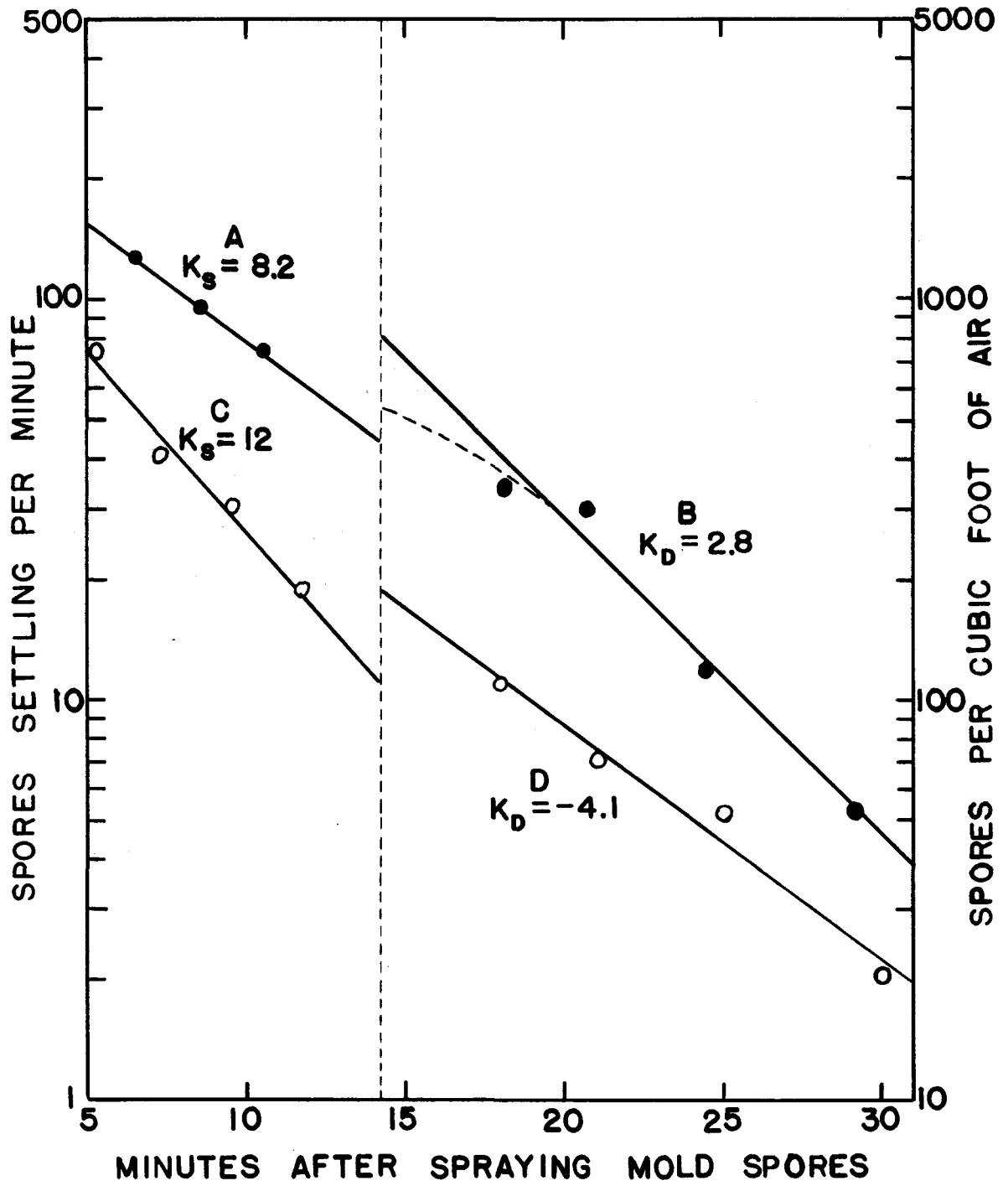


Figure 22. Graphs illustrating the ineffectiveness of TEG vapor against P. notatum spores at 90 per cent relative humidity and 85°F. Lines A and B represent data from sieve collection, while lines C and D are from settling plate data. The dotted line signifies possible lag in killing



effect decreased because of dilution, and that TEG condensed rather slowly because of the scarcity of TEG molecules in the air at high relative humidities. The combined effects would result in the gradual increase in the sizes of the particles thus causing increasingly-rapid settling during the period of observation. In this case one would expect a rapid reversal of the slope of the die-away curve when most of the spores had settled.

Figure 22 illustrates the ineffectiveness of TEG vapor against P. notatum spores at 90 per cent relative humidity and 85° F. Again the symbols are the same as in Figure 19. Note the rapid settling of spore particles in the control period. This is probably caused by reduced evaporation of atomized droplets. The unexpectedly-high sieve counts at line B may be the result of increased efficiency of the sieve samplers for the presumed-larger particles after TEG vapor has condensed upon them, or these data may indicate a partial sigmoid curve such as is commonly found when multi-celled organisms are exposed to lethal agents. The possible lag in killing is indicated by a curved, dotted line. Berry and Michaels (1947) and Jordan and Jacobs (1944), as well as others, have observed sigmoid-type curves when testing the course of liquid disinfection of bacteria when the disinfectant action was weak.

When curves such as line D in Figure 22 were encountered, i.e., where the die-away was less than that of the corresponding control period (resulting in a negative die-away constant), the effect was considered to be a phenomenon of increased settling of particles without apparent killing. Therefore, in order to take advantage of this additional information (correct or incorrect) and also to present estimates of the fungicidal effect of TEG vapors which were conservative (including some recognition of the increased settling of particles having the additional weight of TEG in or on them), the negative die-away constants obtained from settling-plate data in certain experiments have been added to the die-away constants for the sieve data. For example, in Figure 22, in the test period, K_D was -4.1 for settling spores while for spores remaining air-borne K_D was 2.8 indicating a slight killing effect over that of the control period; these two were added giving -1.3 for the K_D reported as the value from the sieve data. The "corrected" value -1.3 suggests that increased settling predominates over killing effects in the removal of viable spore particles from the air.

The experimental results of the effect of relative humidity on the aerosols by TEG vapor have been summarized by means of histograms, (Figures 23 and 24). The widths of the bars designate the relative humidity range which

Figure 23. Histogram showing the die-away effect, K_D , resulting from the action of TEG vapor alone on P. notatum spores at various percentages of relative humidity. These K_D values have been calculated from data obtained by sieve samples. The widths of the bars designate the ranges of relative humidity occurring during the experiments. The bars with the letters A and C immediately above them are averages of two experiments, while the bar designated as B is an average of three experiments

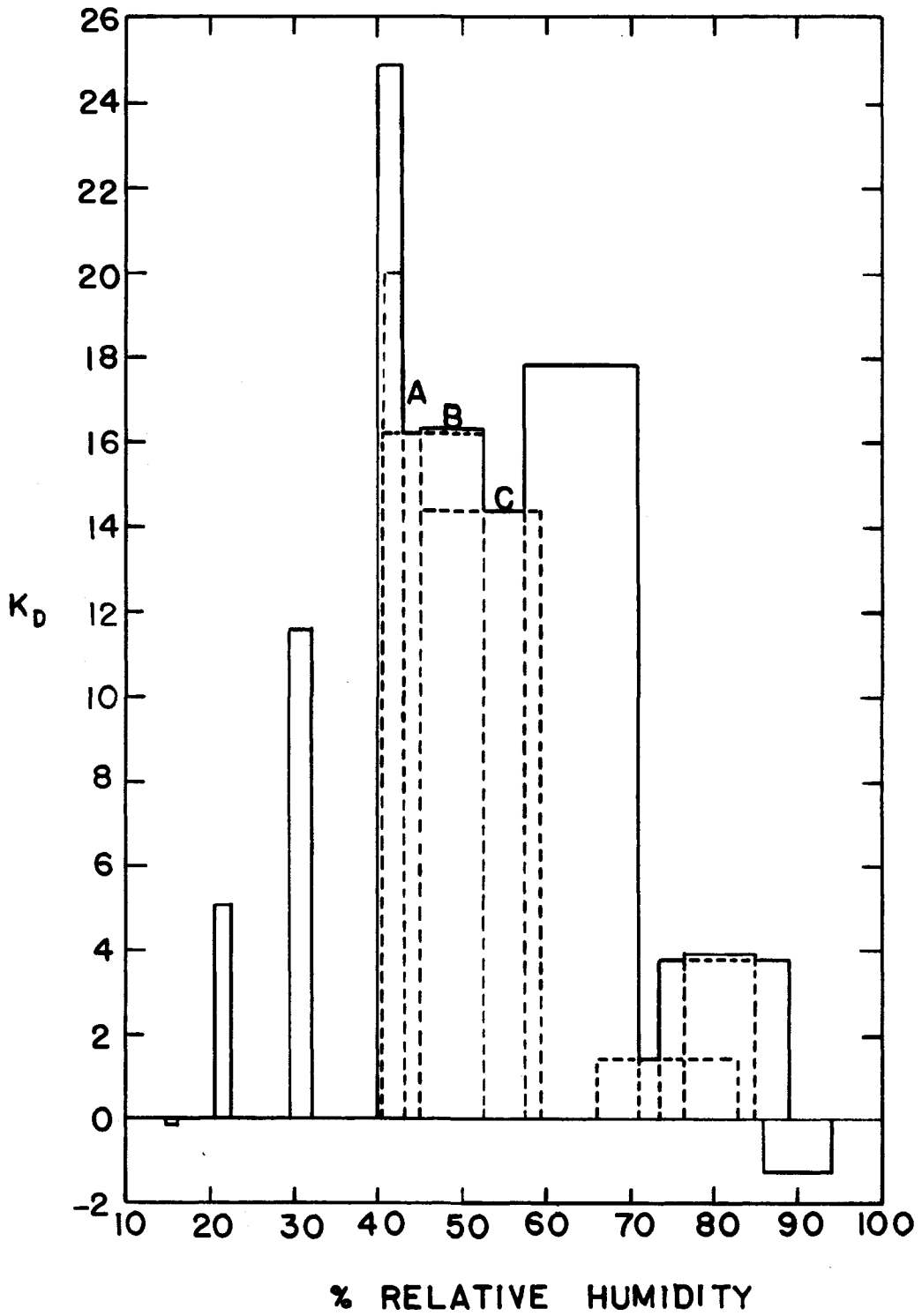
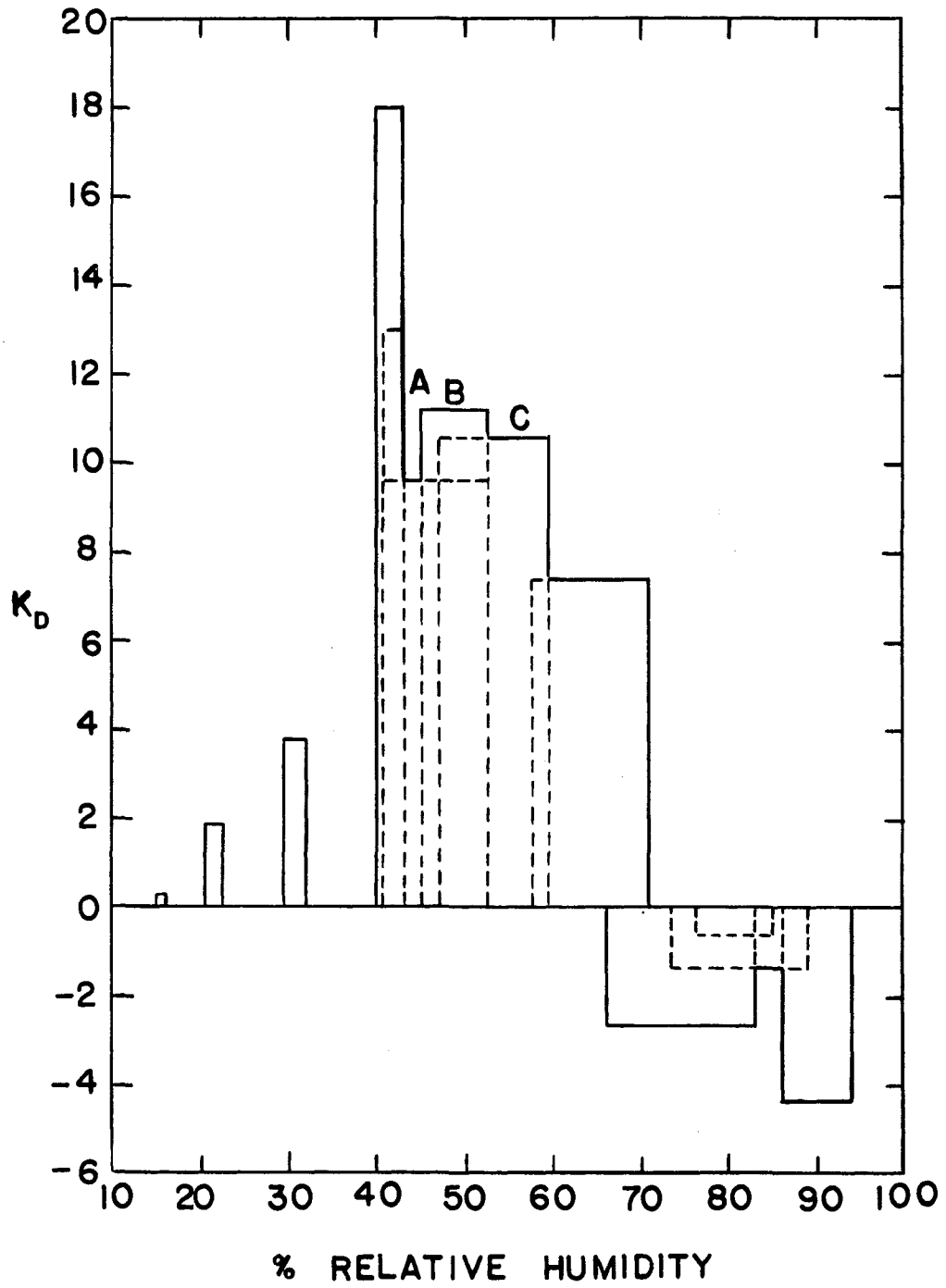


Figure 24. Histogram showing the die-away effect, K_D , resulting from the action of TEG vapor alone on P. notatum spores at various percentages of relative humidity. These values have been calculated from data obtained by settling-plate exposures. The widths of the bars designate the ranges of relative humidity occurring during the experiments. The letters A, B, and C have the same significance as in Figure 22



existed during the tests. Figure 23 shows K_D values calculated from sieve samples. The K_D values have been corrected as indicated above. The bars with the letters A and C immediately above them are averages of two experiments, while the bar designated with the letter B is an average of three experiments. These letters have the same significance in Figures 23 and 24. Note that against particles of smaller mean diameter (i.e., those samples by sieve, Figure 23) TEG vapor in saturated atmospheres was found to be comparatively effective in the relative humidity range of about 35 to 60 per cent. Unfortunately the experiments at the fringes of the effective range were made at the relatively high temperatures of 84 and 85°F (experiments 4 and 12, Table 2) while adjacent experiments in the ineffective range were made at the comparatively low temperatures of 78 and 79°F (experiments 3 and 13, Table 2). Thus the limits of the effective range of percentage relative humidity may be somewhat overaccentuated.

Against particles of larger mean diameter (i.e., those sampled by settling plate, Figure 24) TEG vapor in saturated atmospheres was found to be most effective in the relative humidity range of 40 to 55 per cent. Note that this is a smaller range than that found for smaller particles. Also, as would be expected, TEG vapor did not prove as effective

against the larger particles (Figure 24) as it did against the smaller (Figure 23). The K_D values obtained for the smaller particles were approximately 16 in the effective range, while the K_D values obtained for the larger particles were approximately 10 to 11. These results, except for the magnitudes, are similar to those reported for bacterial particles (Loosli et al., 1947, p. 1393). The series of negative K_D values in the high relative humidity range of Figure 24 might be taken to indicate increased settling with decrease in killing effect.

E. Viability of Spores Exposed to TEG Vapor

The viability of *P. notatum* spores in the presence or absence of TEG vapor in air was tested. The average relative humidity of the air in the test chamber was 45 per cent and the temperature 78°F. The period of suspension of the spores in the atmospheres prior to collection was 10 min for the control and 7 min for the TEG-exposed sample. All spores were collected in sieve samplers and were incubated for 14 hr at 25°C.

Photomicrographs of spores under both conditions were made. It may be seen that in the control sample (Figures 25 and 26) most of the spores have germinated, while in the

Figure 25. Photomicrograph (x 190) of a field of mycelia of P. notatum developed from spore particles collected after a 10-min suspension in air of 45 per cent relative humidity and temperature 78°F. Incubation was for 14 hr at 25°C

Figure 26. Photomicrograph (x 495) of the same culture as in Figure 25 above



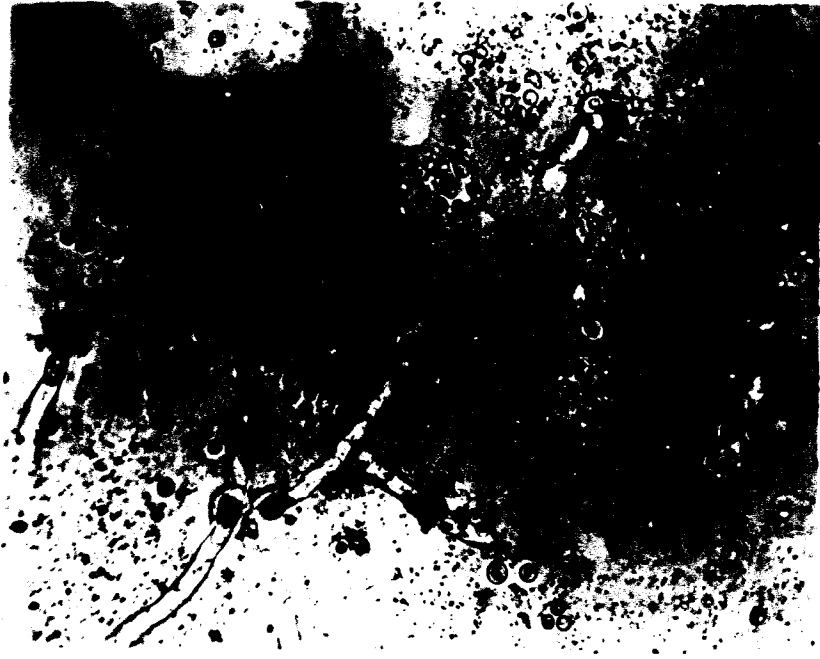
sample exposed to TEG (Figures 27 and 28) only a few have germinated. Spores in the latter are readily identified by their smooth outlines. In a similar experiment in which photomicrographs were not made, it was found by actual count that the percentage of germination in the control test was 93 while in the test using TEG vapor the percentage was 13 after 8 min of exposure. Since the collected spores were deposited in small groups beneath each sieve hole, counting was readily accomplished.

In order to approximate the amount of TEG deposited in the 3 ml of agar on the collection slides during sampling, the aerial TEG concentration at 8 min (obtained from Figure 13) was used to estimate the amount of TEG which passed through the sieve during the sampling period (2 min at 2 cfm). The estimated concentration (0.2 per cent TEG) is far below that exhibiting any inhibitory effects (cf. Mellody and Bigg, 1946, pp. 45-56).

Incubation of collection slides from other experiments, such as those presented in Figures 25, 26, 27 and 28, indicated that spores exposed to TEG vapor for longer periods of time did not germinate after 5 days of incubation on Moyer-Coghill agar.

Figure 27. Photomicrograph (x 495) of a field of spores of P. notatum and several germinated spores developed from spore particles collected after a 7-min suspension in air supersaturated with TEG vapor at 78°F and a relative humidity of 45 per cent. Incubation was for 14 hr at 25°C

Figure 28. Photomicrograph (x 495) of a field from the same culture as in Figure 27. Slightly different lighting was used



F. Possible Decomposition Products of TEG

Because of a report by Gr \ddot{u} n (1950, pp. 291, 292) that the fungicidal action of TEG may be the result of a dissociation of TEG to form formaldehyde, it seemed desirable to investigate possible decomposition products of TEG.

TEG from the original bottle, TEG freshly-distilled under vacuum, and TEG remaining as a residue from the vacuum distillation (temperature did not exceed 105°C) gave positive tests for aldehyde by the Schiff and the Tollen reactions. In the Tollen test a silver mirror was not formed; however, a black precipitate was formed and was considered a positive test. Presence of aldehydes in only trace amounts was indicated since 1 to 2 ml of material were required to produce a positive reaction.

Paper chromatography (solvent: n-heptane saturated with methyl alcohol) of the 2,4-dinitrophenylhydrazine (2,4-DNPH) derivatives formed from carbonyl components present in Air Treatment Grade TEG or its vapors (Glycoaire vaporizer) indicated that formaldehyde was present in TEG fresh from the sealed bottle, in vapors (Glycoaire vaporizer) of TEG taken from the experimental chamber, in vapors arising when TEG was heated in a boiling water bath while bubbling filtered air through it, and in the residual TEG after a

vacuum distillation and replacement of the atmosphere with nitrogen. Because of the nature of the device used for chromatography (chromatocoil) R_f values were not obtained. Precipitates were identified as having a formaldehyde component when spots were present which traveled the same distance as the control formaldehyde derivative.

Since it is rather clear (Wenkert, 1956) that pure TEG will not decompose to form formaldehyde by air oxidation, by heat (as applied to vaporize TEG), or by combination of these conditions, it seems likely that the formaldehyde in Air Sterilization Grade TEG must be there as the result of the manufacturing process. To test this hypothesis, TEG was heated in a boiling water bath and air was bubbled through it and over into 2,4-DNPH solution for 5 hr. The weight of 2,4-DNPH derivatives precipitated in both vessels (after cooling the TEG and adding 2,4-DNPH solution) was 4.25 times as much as that formed in an equal weight of untreated TEG. The greater quantity of precipitate from the heated TEG is probably not the 2,4-dinitrophenylhydrazone of formaldehyde but appears to be other carbonyl compounds formed by dissociation of TEG. Moreover, chromatography of the 2,4-DNPH derivatives indicated the presence of at least five components (in addition to formaldehyde) from the degradation of TEG.

Glycoaldehyde, glyoxylic acid, glycolic acid, and glyoxal were considered as possible simple dissociation and oxidation products of TEG. Derivatives (2,4-DNPH) of glycolaldehyde, glyoxal, and glyoxylic acid were prepared and tested by chromatography with 2,4-DNPH derivatives made from carbonyl compounds present in the various TEG samples mentioned previously. None of the latter was found to compare with glyoxylic acid.

The 2,4-DNPH derivative of glycolaldehyde appeared to be identical with that of glyoxal. This was thought to be the result of the oxidation of the glycolaldehyde by 2,4-DNPH so that a di-2,4-DNPH derivative equivalent to that of glyoxal was formed. The above type of reaction is common with sugars, e.g., where glucose and fructose yield the same osazone (Fieser and Fieser, 1950). Since the 2,4-DNPH derivatives of glyoxal and glycolaldehyde apparently were the same, the formation of the 2,4-dinitrophenylhydrazone could not be used to test for the presence of glycolaldehyde.

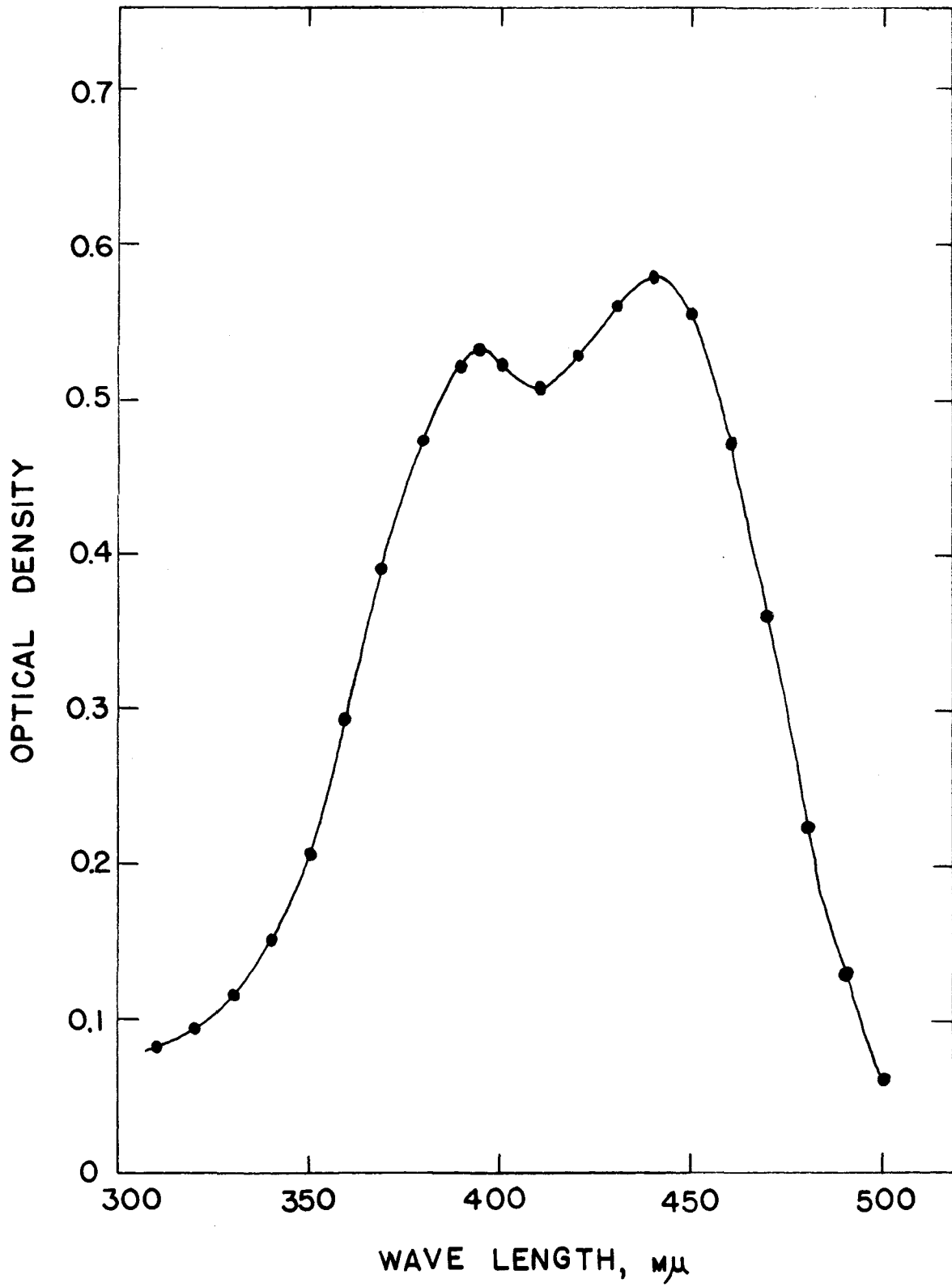
There was a faint spot on the chromatogram indicating the presence of a dicarbonyl compound other than glyoxal when the glycolaldehyde derivative was chromatographed. This compound might possibly have been formed by aldol condensation in the acidic 2,4-DNPH solution.

It has been found (Hammond, 1956) that when 2,4-DNPH derivatives on chromatographic paper are sprayed with potassium hydroxide solution, the spots for the monocarbonyl compounds are generally brown while those for the dicarbonyl compounds are generally blue or purple. In addition, visible and ultraviolet absorption spectra for the 2,4-DNPH derivatives of certain known carbonyl compounds were furnished by Hammond (1956). Use was made of the above information in studies of the 2,4-DNPH derivatives found in all samples of TEG tested.

When paper chromatography with either of the two solvents used in this study was attempted for the known 2,4-DNPH derivative of glyoxal and one of the derivatives prepared from the TEG, no movement in either solvent occurred. Also, both compounds produced a blue spot when the paper was sprayed. In addition, the absorption spectrum obtained from the unknown 2,4-DNPH derivative coincided precisely with that of the known compound (Figure 29). From the evidence at hand, one of the 2,4-DNPH derivatives found from all TEG samples was considered to be glyoxal. However, the glyoxal derivative may very well have originated from a glycolaldehyde component in TEG.

In the present study only formaldehyde and glyoxal (possibly originating from glycolaldehyde because of the method

Figure 29. Absorption spectrum of a 2,4-DNPH derivative of a compound present in vaporized TEG. The solvent was dioxane



used) have been tentatively identified as components of the degradation of TEG. Further work is necessary to establish the nature of other components which appear on the paper chromatograms of the 2,4-DNPH derivatives.

Several compounds which are considered to be possible dissociation and air oxidation products from TEG (i.e., glycolaldehyde, glyoxal, glyoxylic acid, and glycolic acid) were tested as vapors (present in quantities to produce a visible fog) in the experimental chamber for their effectiveness as air disinfectants against spores of P. notatum. These results are summarized in Table 5.

Table 5. Die-away constants obtained when suspected dissociation and air oxidation products of TEG were tested as vapors (present in concentrations sufficient to produce a visible fog) against air-borne spores of P. notatum

Compound tested	Sieve data		Settling plate data		Average temperature (°F)
	K_S^a	K_D	K_S	K_D	
glycolic acid	2.3	about 130	10	51	82
glycolaldehyde	0.64	about 130	9.2	50	77
glyoxal	2.5	20	9.0	18	85
glyoxylic acid	0.95	70	7.5	39	82

^aAll K values are expressed in hours⁻¹.

The K_D values obtained indicate that glycolic acid and glycolaldehyde are five to six times as effective air disinfectants as TEG whose largest K_D values were 20 to 25. Glyoxylic acid was found to be about twice as effective, while glyoxal was found to be of the same order of effectiveness as TEG. Except for glycolic acid all of the compounds listed in Table 5 were vaporized from a steel plate. This was done to avoid possible danger of explosion which could possibly occur by using the electric motor of the glycoaire vaporizer.

Formaldehyde, which was identified as being a component of Air Treatment Grade TEG, is a well-known air disinfectant. Whether there is enough formaldehyde present to account for the effectiveness of TEG vapor is not known.

Precise quantitative studies on the amounts of carbonyl compounds found in TEG vapors were not conducted. However, from crude estimates of aerial TEG concentration during an 8-hr collection of vapors in 2,4-DNPH solution, 0.224 g of precipitate were obtained from approximately 2.2 g. of TEG.

G. Effect on Dry Spore Particles

In a single experiment, dry spores, blown by compressed air from an agar slant, were tested against supersaturated

TEG vapor with a relative humidity of 51 per cent and temperature of 81°F. It was found that the K_S and K_D values for the smaller, sieve-collected particles were 2.2 and 12, respectively, while those for the larger particles (collected by settling plate) were 3.2 and 9.7, respectively. These values indicate that dry spores are more resistant to TEG vapor than spores from aqueous suspensions, the resistance being of the same order of magnitude as the larger, rapidly-settling atomized particles (i.e., $K_D = 10$ to 11).

With both sizes of particles a lag period of 4 to 5 min was observed before killing action was evident. This was contrary to the behavior of moist spore particles. The lag was possibly the result of slower diffusion of TEG into dry particles. A comparison of the behavior of atomized spores and dry spores revealed a rather marked difference in the control-period die-away constants for settling particles. In the former case the K_S values were about 8 to 10 at relative humidities of near 50 per cent, while in the latter instance the K_S was observed to be 3.2. This indicates that the average size of the largest dry-spore particles was only about 5.4μ , while the mean size of the largest atomized particles was found to be about 9.3μ (unit density being assumed for both dry and moist particles). The more limited size distribution of the dry spores is reflected in the

closer K_D values (9.7 and 12) found with collection-plate and sieve samples, respectively, as compared to K_D values of about 10 and 16, respectively, for atomized spore particles.

H. General Discussion

In expressing the rate of removal (die-away) of spore particles from air it has been assumed that the numbers being removed in successive short intervals of time are proportional to the numbers present at the beginning of each interval, i.e.,

$$\frac{dN}{dT} = -kN$$

where N is the number present at time T (in minutes) and k is a proportionality constant. Integrating from N_1 to N_2 and T_1 to T_2 results in

$$\ln N_2 - \ln N_1 = -k(T_2 - T_1) .$$

Converting logarithms to the base 10 and using K to express the die-away per hour provides the form used for calculating the K_S , K_D and K_T values reported herein:

$$K = \frac{138 (\log_{10} N_1 - \log_{10} N_2)}{t_2 - t_1} ,$$

where t_1 and t_2 are in minutes.

Mellody and Bigg (1946, pp. 45-56) have investigated the fungicidal action of TEG vapor on spores of P. notatum, concluding that supersaturated atmospheres of TEG are necessary for optimal killing. The range of relative humidity for most effective killing was given as 45 to 60 per cent. The range found in the present study (35 to 60 per cent) agrees fairly well. However, the results of Mellody and Bigg, which show that supersaturated vapor concentrations are more effective than nearly saturated atmospheres, were not borne out herein.

Puck (1947a, pp. 729-739) has calculated the time required for collision of aerosol droplets and bacterial particles, and also the time required for the collision of vapor molecules (to build to a killing concentration) and bacterial particles. The former apparently would require approximately 200 hr, while the latter approximately 4 sec. While spores of P. notatum studied herein are considerably larger than most bacteria, it is believed that this fact could not be sufficient to greatly alter the general nature of the calculations of Puck. It is thought, therefore, that

the results of the present study (that supersaturated atmospheres of TEG are no more effective than saturated atmospheres) support the computations of Puck.

Using the method of Kaye and Adams (1950, pp. 661-663) for measuring TEG vapor concentration, it was discovered that the particular lot of CP sulfuric acid used made considerable difference in the color reaction. To avoid variation in the color reaction one lot of sulfuric acid was used throughout this work. The reaction was found extremely sensitive to moisture. This was evident by the observation that blowing sulfuric acid from pipettes was found to add enough moisture to influence the results.

Microscopical observations of ungerminated spores indicated that TEG vapor does not rid the air of spores entirely by settling them. The conclusions of Nagy and Mouroumseff (1950, pp. 593-595; 1951, pp. 698-699), that TEG produces its effect only by settling bacteria, stand alone among those of scores of other workers. More information is needed, however, regarding the importance of increased settling in relation to the killing effect. In the present study, simultaneous observation of both settling spore particles and those remaining in the air, and the "correction" (applied from information obtained from settling spores) of die-away

constants attributed to killing was an attempt to distinguish between the settling and killing effects.

The finding by Grün (1950, pp. 291-292) that formaldehyde is present in TEG vapors has been confirmed by the present study. However, it is believed that the formaldehyde does not come from the dissociation of TEG (Wenkert, 1956).

The significance of the dissociation products of TEG and further air oxidation of these compounds, is not known. It is possible that some of them (at least five were indicated by chromatography) may at least contribute to the germicidal or fungicidal action of the vapors. These compounds appear to be present in rather small amounts in the vapors. Before any conclusions as to the significance of the compounds can be made, quantitative studies are needed.

V. SUMMARY AND CONCLUSIONS

The apparent killing rate of air-borne spores of P. notatum by TEG (triethylene glycol) vapor was investigated with respect to relative humidity and vapor concentration. A brief observation of the effect of temperature on the reaction also was made. In addition, the mechanism of action of TEG was investigated with regard to the question of removal of spores from air by settling as opposed to killing. Moreover, the possibility of the mode of action of TEG through degradation products was studied. A static-type of observation (non-continuous air-flow) was used in conducting studies in an experimental chamber.

The average particle size of the spores atomized from aqueous suspension throughout these studies was calculated to be 3.9μ by use of Stokes Law. By collecting these spores, vapor-treated and non-vapor treated, from the air and observing them microscopically after various incubation periods, it was discovered that 87 per cent of spores of P. notatum exposed to TEG vapor for a period of 8 min did not germinate after 14 hr. Upon incubation of spores exposed for periods of 23 min, it was found that in some cases 100 per cent of the spores failed to germinate after 5 days. It was concluded that spores of P. notatum were not merely settled by TEG, but that a killing effect was evident.

Results of vapor concentration studies indicated that at the relative humidities of the test (about 50 per cent) the vapors of TEG were essentially ineffective against spores of P. notatum until about 2/3 saturation was reached. At about 3/4 saturation, the vapor was as effective as at near-saturation or at supersaturation.

On testing relative humidities ranging from 15 to 90 per cent, the effective range of action for TEG vapors against the spores was found to be 35 to 60 per cent relative humidity, with a rapid drop in action outside this range. The most effective relative humidity range at temperatures of 76 to 85°F was 40 to 50 per cent.

The effect of temperature on the action of TEG vapor was investigated by exposing a portion of a spore suspension to the action of supersaturated TEG vapor at 83 and another portion at 105°F. The killing rate at the higher temperature was more than double that at the lower. It should be mentioned, that the increased killing rate at the higher temperature could have resulted, in part, from the increased vulnerability of the spores because of incipient germination (4 hr in distilled water) prior to atomization. The confounding effect of temperature on measurements within the range 76 to 85°F

was thought not to be serious because of the rapid drop in effectiveness at the extremes of the relative humidity range.

Dry spore particles (mean diameter about 5.4μ) of P. notatum were found to be killed by TEG vapor. However, they proved more resistant than spores atomized from aqueous suspension.

In studying the possibility that degradation products of TEG could be the effective fungicidal agents in TEG vapors, it was found that formaldehyde was present in TEG Air Treatment Grade, in vapors of TEG taken from an experimental chamber, in vapors arising when TEG was heated in a boiling water bath while bubbling air through it, and in the residual TEG after vacuum distillation.

The existence of glyoxal (probably derived from glycolaldehyde because of the analytical method used) in TEG vapors or the liquid, even after distilling off half of the material, was demonstrated by absorption spectra and chromatographic behavior of its 2,4-dinitrophenylhydrazone. Other probable dissociation products of TEG or possible products of further air oxidation were observed on paper chromatograms but were not identified. These products consisted of both mono- and dicarbonyl compounds (about four or five in all).

Several compounds which were postulated early in this study as possible degradation products of TEG (i.e., glycol-

aldehyde, glycolic acid, glyoxal and glyoxylic acid) were tested as vapors (present in quantities to produce a visible fog) against spores of P. notatum. Results obtained indicate that glycolaldehyde and glycolic acid are five to six times as effective air disinfectants as TEG. Glyoxylic acid was found to be twice as effective, while glyoxal appeared to be of the same order of effectiveness.

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Figure 1. Phase diagram (reproduced from Puck, 1947b, p. 748) showing the maximum relative percentage of the pure germicide vapor pressure which may exist in relation to the existing percentage relative humidity. The mole-percentage germicide in the condensing vapors is also given

Figure 2. Photograph of the experimental chamber and some of the equipment used for aerial disinfectant studies

Figure 3. Photograph of the experimental chamber showing support on two timbers

Figure 4. Photograph of the top of the experimental chamber showing insulated air cooling ducts including blower at left, refrigerated coils at center, and air filter at right

Figure 5. Photograph of floor of experimental chamber showing fan, with vaporizer beneath, petri dishes, and sieve air samplers at the far end. The rod in the foreground was used for removing petri-dish lids

Figure 6. Photograph of the vaporizer used. Note glass tape running over two pulleys, and heating block to the left and below the top pulley. Reservoir is beneath the lower pulley

Figure 7. Sieve air-sampling device (duBuy and Crisp, 1944, pp. 829-832). "A" is sieve top which mounts onto "C" which contains petri dish. "E" shows the assembled sampler

Figure 8. Photograph of a modified Moulton air sampler (Moulton et al., 1943, pp. 51, 52). Visible from left are the atomizing chamber, bubbling chamber, and Kjeldahl trap

Figure 9. Photograph of three atomizers used. "A" is a De Vilbiss-brand atomizer. "B" is a specially-modified De Vilbiss atomizer, and "C" is a De Vilbiss No. 44 nebulizer

Figure 10. Apparatus used to calibrate air flow-rate. "A", water manometer; "B", tank of water; "C", 5-gal glass jar; "D", sieve air sampler with funnel clamped on top; "E", water trap; "F", flowmeter; and "G", line to vacuum pump

Figure 11. Standard curves obtained for determination of serial TEG concentrations at various concentrations of water by the method of Kaye and Adams (1950, pp. 661-663)

Figure 12. Relationship between the amount of water in a 10-ml, TEG-sulfuric acid sample and the reciprocal of the slopes, K , of the lines showing the relationship between optical density and milligrams TEG in the 10-ml sample (cf. Figure 11)

Figure 13. Graph showing the increase in arial TEG concentration in the experimental chamber during separate experiments when the commercial vaporizer (Glycoaire) was run at full capacity

Figure 14. Graph of the settling of spores as collected by settling plates. The relative humidity was 51 per cent and the temperature 78.5°F. Decrease in settling rate is indicated by the broken line.

Figure 15. Graph showing settling of P. notatum spores in relatively quiet air. The relative humidity was 48 per cent and the temperature 76°F

Figure 16. Graph showing data from Figure 15, except that the plot has now been transferred to semi-logarithmic coordinates

Figure 17. Graph of data from Figure 15, except that the plot has now been transferred to logarithmic coordinates

Figure 18. Broken-line graph showing the approximate relationship between aerial TEG concentration and die-away rate, K_D , of P. notatum spores. The three points at the right of the figure represent results from supersaturated atmospheres. Sieve samplers were used

Figure 19. Graph showing results of a typical test for the fungicidal action of TEG vapor on air-borne P. notatum spores. The dotted line represents the point at which TEG vaporization was started. Lines A and B represent data from sieve collection, while lines C and D are from settling-plate data. Die-away constants are placed beside the lines from which they were derived. The relative humidity and temperature of the experiment were 41.5 per cent and 85°F

Figure 20. Graph showing the ineffectiveness of TEG vapor against P. notatum spores at 15.5 per cent relative humidity and 79°F. Lines A and B represent data from sieve collection, while lines C and D are from settling plate data

Figure 21. Graph of a test of the effect of glycerol vapor against air-borne spores of P. notatum. Symbols have the same significance as in Figure 19. K_T = total die-away resulting from all causes

Figure 22. Graphs illustrating the ineffectiveness of TEG vapor against P. notatum spores at 90 per cent relative humidity and 85°F. Lines A and B represent data from sieve collection, while lines C and D are from settling plate data. The dotted line signifies possible lag in killing

Figure 23. Histogram showing the die-away effect, K_D , resulting from the action of TEG vapor alone on P. notatum spores at various percentages of relative humidity. These K_D values have been calculated from data obtained by sieve samples. The widths of the bars designate the ranges of relative humidity occurring during the experiments. The bars with the letters A and C immediately above them are averages of two experiments, while the bar designated as B is an average of three experiments

Figure 24. Histogram showing the die-away effect, K_D , resulting from the action of TEG vapor alone on P. notatum spores at various percentages of relative humidity. These values have been calculated from data obtained by settling-plate exposures. The widths of the bars designate the ranges of relative humidity occurring during the experiments. The letters A, B, and C have the same significance as in Figure 22

Figure 25. Photomicrograph (x 190) of a field of mycelia of P. notatum developed from spore particles collected after a 10-min suspension in air of 45 per cent relative humidity and temperature 78°F. Incubation was for 14 hr at 25°C

Figure 26. Photomicrograph (x 495) of the same culture as in Figure 25 above

Figure 27. Photomicrograph (x 495) of a field of spores of P. notatum and several germinated spores developed from spore particles collected after a 7-min suspension in air supersaturated with TEG vapor at 78°F and a relative humidity of 45 per cent. Incubation was for 14 hr at 25°C

Figure 28. Photomicrograph (x 495) of a field from the same culture as in Figure 27. Slightly different lighting was used

Figure 29. Absorption spectrum of a 2,4-DNPH derivative of a compound present in vaporized TEG. The solvent was dioxane