Urethanes and Soil Nitrification

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Urethanes have marked physiological activity in plants and animals. Lefevre (1939) found that phenyl urethane affects wheat germination, and subsequently Templeman and Sexton (1946) showed that a variety of urethanes suppress the germination of wheat and oats without affecting chardock. The most active urethanes (for example, isopropylphenylcarbamate, ethylphenylcarbamate) are effective at a concentration of a few parts per million. These arylcarboxylic esters are known to affect mitosis in cells and this fact led to an investigation of urethanes as possible inhibitors of tumor growth. Haddow and Sexton (1946) obtained positive results in animals with isopropylphenylcarbamate, ethylphenylcarbamate and ethyl urethane, though the last is apparently inactive in plants. Ethyl urethane is used in the treatment of leukemia (Patterson et al., 1946), but in mice its administration gives rise to lung tumors (Larsen and Heston, 1945; Larsen, 1947). These effects of urethane take place at much smaller concentrations than those required to bring about its well known narcotic affects. Esterases are inhibited by urethanes (Stedman and Stedman, 1931, 1932), the inhibition of choline esterase by such urethanes as eserine and prostigmine being well known. Schweitzer, Stedman and Wright (1939) conclude that the urethane grouping is essential for esterase inhibition.

Meyerhof (1916) showed that urethanes inhibit the respiration of nitrite oxidising organisms, the inhibitory power increasing with increasing size of the aliphatic radicle; for example, methyl urethane, inhibits the oxygen uptake of these organisms by 50 per cent at a concentration of 0.3M, but isomyl urethane is equally effective at a concentration of 0.022M. Ethyl urethane also inhibits respiration of isolated pure cultures of Nitrosomonas by 42 per cent at a concentration of 0.016M and gives 4 per cent inhibition of Nitrobacter respiration at a concentration of 0.11M. The inhibitory effect of urethane was confirmed by Lees and Quastel (1946), who found, however, a higher sensitivity of the process of nitrification to ethyl urethane in soil culture. The inhibitory effect of ethyl urethane on soil nitrification was also shown to be reversible. Quastel and Scholefield (1949) pointed out that while 0.01M ethyl urethane completely suppresses the nitrification of 0.01M ammonium chloride in garden soil for about twenty days, the subsequent rate of nitrification proceeds normally. The final nitrate nitrogen recovered, however, accounts for 80 per cent of the total nitrogen of the ammonium chloride and of the ethyl urethane. Urethane perfused by itself, is ultimately metabolized by soil organisms. Moreover, urethane at low concentrations (10⁻⁶M) greatly inhibits nitrate formation from ammonium ions on perfusion through a fresh soil, but at a concentration of 3.6 × 10⁻⁶M it inhibits by only 50 per cent the rate of oxygen uptake due to ammonium ion oxidation in an enriched soil. At a concentration one tenth that required to produce a definite inhibition of the rate of oxygen uptake, ethyl urethane secures almost complete inhibition of proliferation of the ammonia oxidising organisms in soil. The conclusion was drawn (Quastel and Scholefield, 1949) that ethyl urethane, at low concentrations, interferes with some aspect of ammonia metabolism essential for the development of these organisms.

Although ethyl urethane at a concentration of 3 × 10⁻⁶M inhibits the growth of the ammonia oxidising organisms, and at 36 × 10⁻⁶M inhibits by 50 per cent their rate of ammonia oxidation, a concentration of 2,000 × 10⁻⁶M is required to inhibit the respiration of rat brain cortex of 50 per cent (Jowett, 1938) and a concentration of 300 × 10⁻⁶M inhibits guinea pig brain respiration by only 20 per cent and does not affect yeast respiration at all (Quastel and Wheatley, 1932).

Quastel and Scholefield (1951) conclude from the results obtained on perfusing mixtures of ammonium chloride and ethyl urethane through soils that nitrification of such mixtures takes place only when organisms capable of breaking down the urethane have developed in the soil.

It is the purpose of this paper to describe the effects of various urethanes on soil nitrification and to show that, in the presence of ethyl urethane, organisms develop in soils which possess an enzyme capable of attacking ethyl urethane probably with the formation of ethanol (cf. Skipper et al., 1951).

METHODS

The technique adopted has been fully described elsewhere (Quastel and Scholefield, 1951) and therefore will not be given in detail. Briefly, the method of investigation was that of continuous perfusion in the dark at 21°C of a neutral solution containing ammonium chloride and/or ethyl urethane through a column of 30 g
air-dried crumbed soil (2–4 mm crumbs) under conditions of optimal aeration and water saturation but with no waterlogging. The rates of conversion of ammonium ions to nitrate were measured, the methods of analysis of samples from the soil perfusion apparatus being those previously described (Quastel and Scholefield, 1951).

Manometric studies were carried out with the conventional Warburg manometric apparatus using soils enriched or saturated with nitrifying organisms or with organisms capable of oxidising ethyl urethane. The process of adapting such soils for manometric investigations has been described in detail (Quastel and Scholefield, 1951).

**Table 1. Effects of varying urethanes (0.0055 M), on rates of nitrification of ammonium chloride (0.01 M)**

<table>
<thead>
<tr>
<th>URETHANE USED IN PRESENCE OF 0.01 M NH4Cl</th>
<th>TIME IN DAYS TO PRODUCE 70 μg/mL OF NITRATE-NITROGEN</th>
<th>ESTIMATED LAG PERIOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>*Isopropyl carbamate C3H7NH-CO-OC2H5</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Ethyl-N-butyl carbamate C3H7NH-CO-OC2H5</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Ethyl carbamate NH2-COOC2H5</td>
<td></td>
<td>38</td>
</tr>
<tr>
<td>Ethyl carbamate NH2-COOC2H5</td>
<td></td>
<td>15.5</td>
</tr>
<tr>
<td>Ethyl - N-isopropyl carbamate (CH3)3CH-NH-CO-OC2H5</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>*Ethyl-2-cyclohexylecyclohexyl carbamate C4H11-C6H14-NH-CO-OC2H5</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>*Octyl-cyclohexylcarbamate C8H17-NH-CO-OC2H5</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>*Octyl-N-phenyl-carbamate C9H18-NH-CO-OC2H5</td>
<td></td>
<td>9</td>
</tr>
</tbody>
</table>

* used in suspension.  
† 30 g soil at 21 C.

**Results**

**Effects of Urethanes on Nitrification of Ammonium Ions in Soils**

A series of urethanes R-NH-CO-OR, including ethyl urethane NH2-CO-OC2H5, was perfused in the presence of 0.01 M NH4Cl through soil. Their effects on the length of the lag period before nitrification of the ammonium ion commenced was determined from the period required to produce 70 μg/ml nitrate-nitrogen. Typical results are shown in table 1.

The most active compounds in the series were ethyl-N-butyl carbamate and isopropyl-carbanilate. Even though the latter was used as a 3.3 × 10^{-4} M suspension it produced a thirty-two-day lag period before nitrification of ammonium chloride began. Ethyl carbamate (urethane) caused an eleven-day lag period at this concentration. The presence of a phenyl group in carbamates is not itself responsible for high toxicity, as ethyl carbaminate produced a shorter lag period (6.5 days) than that due to ethyl carbamate. Ethyl N-isopropyl carbamate produced a 6-day lag period compared with the 32 days obtained with isopropyl carbamate.

**Comparison of Inhibition of Nitrification with Herbicidal Effects of Substituted Urethanes**

Urethanes such as isopropyl carbaminate and ethyl carbaminate, having high herbicidal activities, have marked inhibitory effects on the nitrification of ammonium ions in soil. On the other hand, a powerful inhibitor of soil nitrification, ethyl urethane, has but little herbicidal activity. There is, therefore, no complete parallel between the inhibition of nitrification and the herbicidal action of the urethanes. There may be, however, some connection between these two properties of the urethanes, since the most active of them as herbicides tend to be the most active inhibitors of nitrification. The substituted ureide known as CMU (Cl-C4H10-NH-CO-N(CH3)2), which is a powerful herbicide, is also a powerful inhibitor of soil nitrification. Typical results with CMU are shown in table 3.
Nitrate Formation from Urethanes

Ethyl urethane and other carbamic esters are converted to nitrate in fresh soil after lengthy lag periods. It has already been shown (Quastel and Scholefield, 1949) that urethane may be so converted, and the results given in figure 1 show the effects of perfusing soil with this confined to ethyl urethane. Each value is the average of the results of twelve experiments carried out at the same time.

![Graph](image)

**Fig. 1.** The nitrification of 0.01 m ethyl urethane by enriched soils. Each value is the average of the results of twelve experiments carried out at the same time.

Possibly due to the action of heterotrophic organisms in utilizing the liberated ammonia or its oxidation products. The final rate of nitrification is quite constant during successive perfusions and, once established, may be used for kinetic studies.

The effects of perfusing substituted urethanes alone and in admixture with ethyl urethane over soils enriched by successive perfusions of ethyl urethane are shown in table 4. None of the substituted urethanes studied was immediately converted to nitrate at a rapid rate by the ethyl urethane enriched soil. Thus organisms which develop in soil in response to the presence of ethyl urethane and which are able to attack it, giving rise to nitrate, are not immediately capable of converting to nitrate the other urethanes investigated. On the other hand the presence of various urethanes (for example, isopropyl N-phenyl carbamate, ethyl-N-butyl carbamate, and ethyl-N-isopropyl carbamate) greatly inhibits the rate of nitrate formation from ethyl urethane (table 4).

The mechanism of this inhibition is not yet established. The inhibitory urethane may act by competing with ethyl urethane for the enzyme responsible for the initial attack on this substance, or it may act by inhibiting the nitrification of ammonium ions liberated on breakdown of ethyl urethane. If the first explanation is correct, then the enzyme first attacking ethyl urethane possesses a high degree of specificity. If the second explanation is correct, it is apparent that nitrifying organisms may retain their ability to oxidise ammonium ions in presence of excess of ethyl urethane but not in presence of the other urethanes investigated. Thus an

### Table 4. Effects on rates of nitrate formation of perfusing substituted urethanes (R·NH·COOR') together with ethylurethane (NH₂·COOCH₃) through a soil,† enriched with organisms capable of converting ethyl urethane to nitrate.

<table>
<thead>
<tr>
<th>R</th>
<th>R¹</th>
<th>Rate obtained with ethyl urethane alone</th>
<th>Rate obtained with R·NH·COOR' alone</th>
<th>(2) as percentage of (1)</th>
<th>Rate obtained with mixture of ethyl urethane + urethane (3)</th>
<th>(3) expressed as percentage of (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>C₃H₅</td>
<td>1.405</td>
<td>1.465</td>
<td>104</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C₂H₁₁·C₆H₁₉</td>
<td>C₃H₅</td>
<td>1.016</td>
<td>0.966</td>
<td>98</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C₆H₁₃</td>
<td>C₃H₅</td>
<td>1.598</td>
<td>0.076</td>
<td>5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C₆H₁₃</td>
<td>(CH₃)₂CH</td>
<td>1.669</td>
<td>—</td>
<td>—</td>
<td>1.606</td>
<td>102</td>
</tr>
<tr>
<td>C₆H₁₃</td>
<td>(CH₃)₂CH</td>
<td>1.882</td>
<td>0.037</td>
<td>2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C₆H₁₃</td>
<td>(CH₃)₂CH</td>
<td>1.195</td>
<td>—</td>
<td>—</td>
<td>0.552</td>
<td>4</td>
</tr>
<tr>
<td>C₆H₁₃</td>
<td>C₂H₁₇</td>
<td>0.956</td>
<td>0.053</td>
<td>5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C₆H₁₃</td>
<td>C₂H₁₇</td>
<td>1.243</td>
<td>—</td>
<td>—</td>
<td>0.762</td>
<td>61</td>
</tr>
<tr>
<td>C₆H₁₂</td>
<td>C₂H₁₇</td>
<td>1.405</td>
<td>0.045</td>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C₆H₁₂</td>
<td>C₂H₁₇</td>
<td>1.511</td>
<td>—</td>
<td>—</td>
<td>1.728</td>
<td>114</td>
</tr>
<tr>
<td>C₆H₁₂</td>
<td>C₂H₁₇</td>
<td>2.032</td>
<td>0.091</td>
<td>4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C₆H₁₂</td>
<td>C₂H₁₇</td>
<td>1.357</td>
<td>—</td>
<td>—</td>
<td>1.332</td>
<td>98</td>
</tr>
<tr>
<td>C₆H₁₂</td>
<td>C₂H₁₇</td>
<td>1.203</td>
<td>0.033</td>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C₆H₁₂</td>
<td>C₂H₁₇</td>
<td>1.364</td>
<td>—</td>
<td>—</td>
<td>0.025</td>
<td>2</td>
</tr>
<tr>
<td>C₆H₁₂</td>
<td>C₂H₁₇</td>
<td>1.950</td>
<td><strong>&gt;0.65</strong></td>
<td>&gt;33</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>C₆H₁₂</td>
<td>C₂H₁₇</td>
<td>1.473</td>
<td>—</td>
<td>—</td>
<td>***&gt;0.85</td>
<td>*88</td>
</tr>
</tbody>
</table>

* i.e. Value found on perfusion.  ** Lag period = 6 days.  *** Lag period = 6.5 days.
† 30 g soil. Duration of experiment 8 days at 21 C.
‡ Rates expressed as mg NO₃⁻N formed per ml perfusate per hour.
acquired resistance to ethyl urethane would not imply an acquired resistance to other urethanes.

Course of Breakdown of Ethyl Urethane by Soil Microorganisms

The breakdown of ethyl urethane, on perfusion through soil, is a biological process. This is shown by the fact that the lengthy lag period preceding the conversion of urethane to nitrate is shortened by the introduction into a fresh soil of a small quantity of a soil capable of rapid breakdown of ethyl urethane. The effects on the rates of nitrate formation from ethyl urethane of such inoculation with varying quantities of enriched soil are shown in figure 2, where the logarithm of nitrate-N production on perfusion of ethyl urethane through soil is plotted against time. It will be seen that there is a linear relationship, a result to be expected if the nitrate formation is due to proliferation of microorganisms (Quastel and Scholefield, 1951). Further, the initial rates of nitrate formation are increased when the size of the inoculum of enriched soils is increased and the various lines in figure 2 are parallel to each other. The last fact indicates that the rate of proliferation of the nitrifying organisms is a constant under the experimental conditions.

Manometric Results

Some indication of the mode of breakdown of ethyl urethane in soil is obtained from a manometric study of soils enriched with organisms capable of forming nitrate from this substance. Such soils are obtained by repeated perfusion of a soil with ethyl urethane (0.01M) until constant rates of nitrification are obtained.

The rates of oxygen uptake by enriched soils in the presence of ammonium chloride and of various quantities of ethyl urethane are given in figure 3. The rates of oxygen consumption are greater with urethane than those obtained with an approximately equal or larger concentration of ammonium chloride. This is to be expected if the oxygen uptake due to the combustion of the organic moiety of ethyl urethane is additive to that

![Figure 2](image_url)

**Figure 2.** The effect of size of the inoculum of enriched soil on the rate of nitrate formation from 0.01M ethyl urethane in a fresh soil.
- ○ 1.0 g Inoculum
- ■ 2.0 g Inoculum
- □ 4.0 g Inoculum
- △ No inoculum, showing values obtained at 26th and 28th days.

![Figure 3](image_url)

**Figure 3.** Oxygen uptakes due to the addition of various substrates to a soil enriched with ethyl urethane decomposing organisms. Final volume of fluid added to 1.5 g wet soil is 2.0 ml in all cases.
- ○ 1.0 ml 0.011M Ethyl urethane added.
- ■ 0.5 ml 0.011M Ethyl urethane added.
- □ 0.25 ml 0.011M Ethyl urethane added.
- △ 1.0 ml 0.011M Ammonium chloride added.

**Table 5. Oxygen consumption at 57 C by 1.5 g soil, enriched with organisms converting ethyl urethane to nitrate.**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Substances added to the soil</th>
<th>Time</th>
<th>µl O2 taken up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0 ml water</td>
<td>1</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>1 ml 0.011 M ethyl urethane + 1 ml water</td>
<td>1</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td>1 ml 0.011 M ethanol + 1 ml water</td>
<td>1</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>1 ml 0.011 M ethanol + 1 ml 0.011 M ethyl urethane</td>
<td>1</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td>1 ml 0.011 M acetaldehyde + 1 ml water</td>
<td>1</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>1 ml 0.011 M acetaldehyde + 1 ml 0.011 M ethyl urethane</td>
<td>1</td>
<td>142</td>
</tr>
<tr>
<td>2</td>
<td>2.0 ml water</td>
<td>2</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>1.0 ml 0.011 M ethyl urethane + 1.0 ml water</td>
<td>2</td>
<td>265</td>
</tr>
<tr>
<td></td>
<td>1.0 ml 0.01 M ethylamine (neutral) + 1.0 ml water</td>
<td>2</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>1.0 ml 0.01 M ethylamine + 1.0 ml ethyl urethane</td>
<td>2</td>
<td>223</td>
</tr>
</tbody>
</table>
due to oxidation of liberated ammonia. The optimal rate of oxygen consumption with ethyl urethane is obtained with a concentration of 0.005m.

Using low concentrations of ethyl urethane it is possible to estimate the amount of oxygen consumed for the conversion to nitrate of 1 mol ethyl urethane. For example, the oxidation of 1.0 ml 0.001m ethyl urethane required 68 μl oxygen. This corresponds to an uptake of 6 atoms oxygen per mole ethyl urethane and is in accordance with the following equation:

\[
\text{NH}_2\text{-COOC}_2\text{H}_5 + 3\text{O}_2
\]

\[
= \text{CO}_2 + \text{HNO}_3 + \text{CH}_3\text{COOH} + \text{H}_2\text{O}
\]

A likely method of breakdown of ethyl urethane would be a preliminary hydrolysis to ethanol, ammonia and carbon dioxide, followed by oxidation of ethanol to acetic acid and of ammonia to nitric acid. If this mode of breakdown takes place, then ethanol should be oxidised to acetic acid by a soil enriched with ethyl urethane decomposing organisms. The results shown in table 5 indicate that ethanol is oxidised by such an enriched soil at a greater rate than is ethyl urethane, and this rate is not increased on admixture of ethyl urethane with ethanol. Moreover, the oxygen consumption found with ethanol corresponds to two atoms oxygen per mole of ethanol or the amount required for oxidation to acetic acid. Addition of sodium acetate to an enriched soil leads to little or no increased rate of oxygen uptake.

The addition of acetaldehyde, at concentrations similar to those used with urethane and ethanol, also leads to an increased rate of oxygen uptake, the rate being about that obtained with urethane and is not increased on addition of urethane (table 5). The amount of oxygen consumed amounts to one atom of oxygen per mole acetaldehyde which again corresponds to acetic acid formation.

Thus the rates and amounts of oxygen consumed by ethanol and acetaldehyde in presence of soils enriched with organisms oxidising ethyl urethane are in accordance with the view that the urethane undergoes a preliminary hydrolysis to ethanol which is then oxidised to acetic acid.

It is unlikely that ethyl urethane first undergoes decarboxylation to ethylamine, since the addition of ethylamine to an enriched soil does not lead to any increased rate of oxygen consumption (table 5).

ACKNOWLEDGMENTS

We are grateful to the Monsanto Chemical Company for gifts of the various urethanes used in this investigation. We are also much indebted to the National Cancer Institute of Canada for a grant-in-aid.

SUMMARY

The inhibitive effects of a series of urethanes on the nitrification of ammonium ions in fresh soil have been investigated. The most active compounds of the series are ethyl-N-butyl carbamate and isopropyl carbamate. Eserine is also highly effective, being ten times more active than ethyl urethane.

The ureide, Cl·(CH₂)₂·NH·CO·N(CH₃)₃, known as CMU is a powerful inhibitor of soil nitrification.

Although there is no strict parallelism between herbicidal effects and inhibition of soil nitrification by urethanes, there is a tendency for the most powerful herbicides to have the most vigorous inhibitive effects on nitrification in soil.

Ethyl urethane and other carbamic esters are converted to nitrate in fresh soils after lengthy lag periods.

Organisms that develop in soil in response to the presence of ethyl urethane and are capable of attacking it, are not immediately capable of attacking other urethanes. The presence of a variety of urethanes inhibits the rate of nitrate formation from ethyl urethane.

Manometric studies of soils enriched with organisms attacking ethyl urethane indicate that such organisms attack ethanol and acetaldehyde. The amounts of oxygen consumed for the oxidation of ethyl urethane, alcohol and acetaldehyde are in accordance with the conclusion that acetic acid is the end product. Acetic acid is apparently not oxidised. Kinetic and stoichiometric evidence lead to the conclusion that ethyl urethane is first broken down by soil organisms to ethanol, ammonia and carbon dioxide, with subsequent formation of acetic and nitric acids.

REFERENCES


Humidity Requirements for Mold Growth

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In the warm, humid areas of the United States and of the world, mold or mildew prevention ranks as a major pest-control problem. Leather goods, clothing, foods, mattresses, painted walls, and even glass camera lenses are subject to the unsightly appearance, stain, damage, or musty odor associated with the growth of the lower-order plants belonging to the fungi and known popularly as mildew or mold.

As has been mentioned previously in a discussion of the mildew problem (Block, 1946), mold requires for growth and proliferation certain essential physical and chemical conditions. These include satisfactory temperature, adequate moisture, sufficient oxygen, proper pH, and essential nutrients. Prevention of mold growth may be effected by restricting any of the above requirements. Mildew may be controlled also by physical agents that destroy the organism, such as ultraviolet radiation and heat sterilization, and by chemical poisons called fungicides. The specific conditions best determine the method to be employed to prevent mold growth. In certain applications the control of moisture through regulation of humidity has proved to be most satisfactory (Block, 1951). This applies to the prevention of mildew in home closets, rooms, storage spaces and shipping units.

At the request of the British Public Record Office, Groom and Panisset (1933) studied the conditions necessary for mildew to develop on materials used for book covers. Working with Penicillium chrysogenum, they noted that the minimum relative humidity for spore germination on glass was 81 per cent and for mildewing of leather and other book materials was 72.8 per cent. Since it is obvious that mildew could not be present unless the mold spores could germinate, Groom and Panisset suggest that the spores germinate at a lower relative humidity on book materials because of the presence of nutrients. Galloway (1935) reported that the minimum relative humidity permitting growth of molds varies from 75 to 95 per cent for different species; thus protection of materials is assured only if the atmosphere is below 75 per cent R.H. (relative humidity). Clayton (1942) found that some fungus spores germinate at 0 per cent humidity on glass, but these fungi are associated with plant diseases and not mildewing of inanimate objects. A report of the U. S. National Bureau of Standards (1947) states that at 85 per cent R.H., or less, no mildew growth on leather occurred, but that at 95 per cent R.H. there was heavy growth. Illman and Weatherburn (1947) made an excellent study of the factors affecting the development of mold on various materials. They found no mold growth at 60 per cent R.H. and very little at 70 per cent R.H., but increasing amounts from 80 to 100 per cent R.H.

To what extent does the moisture in the substrate determine mold growth as apart from the relative humidity of the atmosphere? How are different materials affected under the same physical conditions of humidity and temperature? Galloway (1935) offered data to show that atmospheric moisture is more effective than moisture in the substrata for bringing about the germination of mold spores. For information on the second question, Smith (1942) states, “If dry samples of pure wool and pure cotton are exposed to the same atmosphere the wool will take up approximately twice as much moisture as the cotton and, leaving out of account differences due to chemical composition, the two samples will be approximately equally liable to mildew.”