first part of this letter would be expected to form in Tager's process.

Furfural enters into many different types of reactions. One wonders if anyone is able to comprehend all of the possibilities and all of the conditions that might tend to alter the courses of the reactions. We can be sure, however, that some of the products of the reaction between furfural and aniline will be large organic cations.

Microbiological Factors in Soil Stabilization

P. C. T. JONES, Rothamsted Experimental Station,
Harpenden, Hertfordshire, England

Introductory Remarks by the Chairman: It is a maxim in the carbon economy of the biosphere that all carbon compounds formed must be destructible in order to avoid carbon starvation and to maintain life as we know it. A great role in this destruction is played by bacteria and fungi. During the last 20 years the probable contribution by microörganisms to destruction under certain conditions of stabilized soils and of other construction materials containing organic matter has been mentioned a number of times in the engineering literature. But this awakened little interest and less reaction among highway engineers.

The problem became acute, however, during World War II, when airfields successfully waterproofed by means of small amounts of natural resin derivatives returned to their previous unstabilized condition within relatively short periods of time. It was on that occasion that the Road Research Laboratory in Great Britain sponsored a thorough study of this problem at the famous Rothamstead Agricultural Experiment Station. The man entrusted with this investigation was Jones, who did a beautiful job in identifying and isolating the microbial culprits and in exposing their working methods.

Jones, who has long since left the field of soil stabilization research, has taken time out from pressing and important medical research to give us an account of the important role microbiology plays in certain types of soil stabilization.

● IN recent years, interest in the problem of soil stabilization has arisen in two seperate contexts: (1) on account of the intimate relation between soil structure and the important problem of erosion (and in relation to the water and nutrient supply to the living plant) and (2) on account of its relationship to civil-engineering problems of road construction.

In both these cases, variations in the microflora (and microfauna) of the soil will occur and will be of particular importance when foreign readily decomposable materials (such as the resins currently used) are added. The use of cohesive soil materials for construction purposes is of considerable antiquity. Adobe construction is still widely used in the world, even at the present, and (as archaeological findings disclose) was of yet greater importance in times past.

One important feature of the soil and mud materials used was soon established: under wet conditions, mechanical strength is soon lost. Cohesive soils will carry a heavy load as long as they are dry, but when they become wet, the individual soil particles become surrounded by a water film which acts as a lubricant between the platy surfaces of the soil clays and so renders them susceptible to shearing motion, and the soil degenerates into mud.

If the pressure applied to such a soil by a heavy load is spread over a large area, as with the concrete mattress frequently used in road construction, sufficient support is present. Otherwise, depression of that area will occur on account of the plasticity of the soil.

We are all familiar (in Conan Doyle at least) with the bog of legend, which when dry will support a heavy load, but when wet offers a tempting hazard to the novelist and the unsuspecting. This, of course, is an extreme case of the phenomenon of clay lubrication as outlined above.

Again, it is apparent that such soils, which collapse under conditions of high precipitation are of little agricultural value, as well as being readily eroded. If, however, some means are found for rendering these soils water-stable, the solution of both these problems becomes possible.

The stability of natural soils of good structure is maintained by the cohesive action of the so-called humic acids (34), and a greatly enhanced stability can be achieved by the addition of natural or synthetic resins.

It is important here, however, to distinguish between agricultural and civil-engineering requirements. In road construction the object of stabilization is to prevent, as far as possible, the movement of water within the soil once it has been compacted. However, in agricultural practice, the object of such immobilization is conservative and the maintenance of a structure that allows sufficient aeration for the respiring roots of the living plant. Thus, materials such as gum rosin and Vinsol, which can be used for road- or airstrip-stabilization are unsuitable for agricultural purposes; whereas materials such as Krilium are admirable for the latter purpose.

In road construction the soil foundation must be maintained at some relatively constant moisture content at which the soil possesses adequate mechanical strength (stability). This is achieved by various means, such as cementing the soil particles together by binders of some sort, or maintaining the natural cohesion of the soil by waterproofing it.

Substances capable of effecting these ends are known. In the first case, cement and bitumen are well known, familiar in the case of large aggregates as concrete and macadam respectively; and in the latter case, recent research has indicated the use of various compounds, generally possessing a free polar group attached to another, nonpolar in character, which have the property of minimizing the tendency of the soil moisture to increase under wet conditions.

One group of these includes certain natural resins, effective when added to the soil in powder form in quantities of the order of 1 percent by weight. In soil-microbiological studies, it almost goes without saying that whatever material one adds to a soil under natural conditions there will be present among the tremendous diversity of soil microörganisms some that will be capable of attacking and decomposing the material added. Indeed, were this not so, the surface of our globe would be much more encumbered with the dead bodies and waste products of past creatures. Indeed, it is only the casts, calcareous or silicious skeletons or the more resistant lignin-like materials that remain.

It is to this process of ubiquitous decomposition that we owe our coalfields and, in all probability, our oilfields. It is almost a sine qua non that energy-rich materials, such as gum rosin, will be decomposed; and at an early stage it was suspected that they might be susceptible to microbial attack in the soil.

REVIEW OF LITERATURE

Although little work is extant on the microbial attack on natural or synthetic resins, a considerable literature exists on the decomposition of chemically related compounds. The decomposition of hydrocarbons, both aliphatic and aromatic, has been extensively studied. If the views on biogenesis offered by the Russian Biochemist Oparin are accepted (23), it would appear that the ability to metabolize this type of compound is primitive indeed. From his reasoning, based on stellar and planetary spectra and on petrological examination, it would appear that compounds of this type were widespread in his suggested original anaerobic environment.

The available literature on hydrocarbons is well reviewed by ZoBell (41) and by Bushness and Haas (4). Microbial attack on methane was first demonstrated by Söhngen (27). Aiyer (1) demonstrated the use of this hydrocarbon in swamp water by fluorescent rods. Söhngen (28) showed that mycobacteria and pseudomonads were capable of metabolizing gasoline, kerosene, and paraffin wax; while Baldwin (3) showed that the soil flora was modified by the addition of crude petroleum. Büttner (5) used paraffin wax to isolate saprophytic mycobacteria.

Hopkins and Chibnall (16) described a strain of Aspergillus versicolor which would metabolize odd- and even-numbered paraffins up to a chain length of C_{34}; however, they were unable to establish the means of breakdown beyond suggesting the possible occurrence of a polyketone as an intermediate to shorter-chain fatty acids. (It is of interest to note that the present author has isolated a strain of this fungus, which is an active resin-attacker, and has demonstrated the presence of carbonyl compounds in ethereal extracts of culture fluid).

Haag (12) correlated the use of paraffin wax with a decrease in its iodine number, while Grieg-Smith showed that paraffin was readily metabolized if adsorbed on a particulate surface, such as kieselguhr, while the importance of solid surfaces has been justly emphasized by ZoBell (40).

In a natural soil, an extremely large surface is available, and if the substance to be decomposed has some surface activity, such as the resins used in soil stabilization, then a mono- or poly-molecular film is quickly spread over the surface of the soil clays (the process known as "curing") and ideal conditions for decomposition occur.[1]

Attack on aromatic hydrocarbons has been demonstrated by many authors. Störmer (31) demonstrated the microbial decomposition of toluene and xylene, Tattersfield (35) and Jacobs (18) that on naphthalene. The latter author demonstrated the occurrence of phthalic acid in culture filtrates. Strawinski and Stone (32) showed one main fraction to be orthosalicylic acid; they also showed that bacteria were capable of metabolizing such diverse hydrocarbons as cetane, naphthalene, biphenyl, a-methyl naphthalene, and tetralin.

Sen Gupta (24) noted the disappearance of phenol and cresol from soils under natural conditions, while Gray and Thornton (11) successfully demonstrated bacterial attack on naphthalene, phenol, and cresols and found such bacteria to be present in 146 out of 245 soils tested. Wagner (37) isolated from soil and faeces bacteria that could attack phenol, catechol, and phloroglucinol, while Fowler, Ardern, and Lockett (10) suspected the occurrence of phenol attackers in sewage beds. More recently, Happold (13) isolated these organisms from many sources, including sewage.

Happold and Key (14) also isolated a gram-negative vibrio from gasworks liquor, which attacked phenol. Allen, Naghski and Hoover (2) recently studied the decomposition of the resins associated with the retting of the Guayule Shrub (Parthenium argentatum) in order to obtain the dispersed rubber latex of that plant. They observed that the process of retting patented by Spence (29) led to a diminution in the acetone soluble resinous material associated with the rubber, and they described a number of bacteria and fungi that would readily attack the resin, one identifiable as a cresol-attacker isolated by Gray and Thornton, (11). The microbiological decomposition of cholesterol, another closely related compound, has been noted and investigated by Turfitt (36).

Hence, from a survey of the available literature, there is considerable reason to assume that natural resins would be decomposed by soil microörganisms. This belief is sustained by a consideration of the amazing adaptability of these forms of life, c.f., Dubos (7), and from the fact that large quantities of natural resins have not (with the possible exception of amber) accumulated on the surface of the earth.

Since soil conditions will obviously affect the microörganisms concerned, some reference may perhaps be made here to papers on that extensive subject. An encyclopaedic work on environmental factors is that of Van Suchtelen (30) who showed that microbial activity (as expressed by CO_2 evolution) showed an increase with soil moisture content from a minimum at about 4 percent to a maximum at 15 percent. This would seem to indicate that a partial breakdown of resin stabilization would only serve to accelerate this process.

However, on the other hand, Hubbell and Gardner (17), in a paper most pertinent to the present problem, showed that soil compaction produced a considerable reduction in both aggregation and soil flora, both of these showing some correlation with the noncapillary pore space. From this it would seem that compaction itself tends to reduce microbial attack, an indication which has also been given in the present author's results.

Engberding (8) and Skinner, Jones, and

[1] Note by H F W Whether adsorption on a solid surface makes an attack easier or more difficult depends on whether the susceptible parts of the molecules are directed towards, or adsorbed by, the solid surface or are on the exposed side of the adsorbed layer (see paper No 6, by Gieseking)

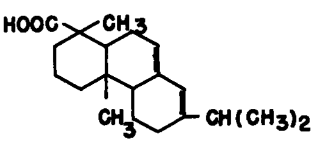
Mollison (26) have shown correlations between moisture content and bacterial numbers, the former using the direct count technique of Jones and Mollison (20), in addition. Winterkorn (39) noted effects of microbial activity on the strength of earth embankments and of bitumen-treated calcium soils.

It seems that there is considerable reason to assume that natural resins are decomposed in soils and that modifications of the local physical conditions materially affect the nature of the attack. The following material describes some aspects of this work and attempts to relate it more particularly to the practical problems of soil stabilization and road construction.

The practice of stabilizing, or more simply, waterproofing, road foundations with resins was initiated during the late war, and was initially developed for military purposes. When the resinous compounds used were incorporated into soil, the mixture resulting (ca. 1 percent resin) was compacted by rolling in order to obtain a mechanically substantial foundation. It was also realized that an optimum moisture content existed for each soil treated, and that the presence of excess (more than about 5 percent) of organic matter was deleterious to successful stabilization. Moreover, frequent failures occurred which were not readily ascribable to any of the known physical variables. It was then realized that some other, possibly biological, factor was involved. This article is intended to indicate how such factors may be elucidated and what problems they subsequently pose; with an indication to the most likely means of their resolution.

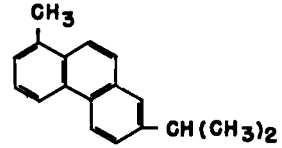
In this work, the three resinous materials investigated were (1) Vinsol, manufactured by the Hercules Powder Company, Wilmington, Delaware., (2) the natural residue from oleoresin distillation (viz, gum rosin), the principal constituent of which is abietic acid ($C_{20}H_{30}O_2$) and its isomers; and (3) "321" resin, being a proprietary mixture of gum rosin and sodium rosinate in the proportion of 3 to 1.

The work of Ruzicka and of Fieser has demonstrated the most-likely formulas of abietic acid to be



the rings being saturated with the exception of the double bonds indicated.

This is manifestly related to the aromatic hydrocarbon retene



which is methyl-isopropylphenanthrene.

It will be seen that abietic acid is monobasic, and by virtue of its hydrophobic abietyl group forms a mono- or poly-molecular film on a water surface, a free-surface energy of about 10 dynes per centimeter being available. However, the sodium salt, by virtue of the high hydration capacity of the sodium ion, will be soluble at an alkaline pH. But the free acid and alkaline earth salts both form mono- or poly-molecular rigid films, which later can easily be demonstrated by powdering a surface film of resin on a water surface with talc when the whole surface is seen to move on disturbance as a single rigid unit.

Vinsol is an extract of pine stumps made with an organic solvent and contains some abietic acid in addition to other terpenelike materials. It will be seen below that non-carboxylic ^-OH is present, the acid number being smaller and the molecular free energy less. A summary of the salient chemical and physical characters of these compounds is set out below. (From Appendix B. Cement-Durability Program, First Interim Report, War Department, U.S. Corps of Engineers, U. S. Army, Mount Vernon, New York, June, 1942-).

A theory of the stabilization of soils based on the formation of a surface film in the soil moisture has been developed by Clare (6) and others, who have demonstrated effective stabilization below pH 7, failure near neutrality, and film formation (presumably of the calcium salt) at pH values higher than 7.5.

METHODS OF INVESTIGATION

As few techniques were available for the investigation of this problem of microbial attack on resins incorporated in natural soils, the development of new and modified techniques seemed essential. These comprised: - (1) methods of isolating resin-attacking bacteria; (2) a method for selectively counting these bacteria; (3) methods for the detection and approximate estimation of resins in soil; and (4) a standard method of studying water absorption as used at the Road Research Laboratory, Harmondsworth, Middlesex, England.

Several other lines of inquiry of a more strictly biochemical nature were pursued, such as the study of resin attack by pure cultures of bacteria by micromanometric methods. But since these form the subject of another paper and are not strictly relevant to the immediate problem of the engineer, they are not dealt with at length below.

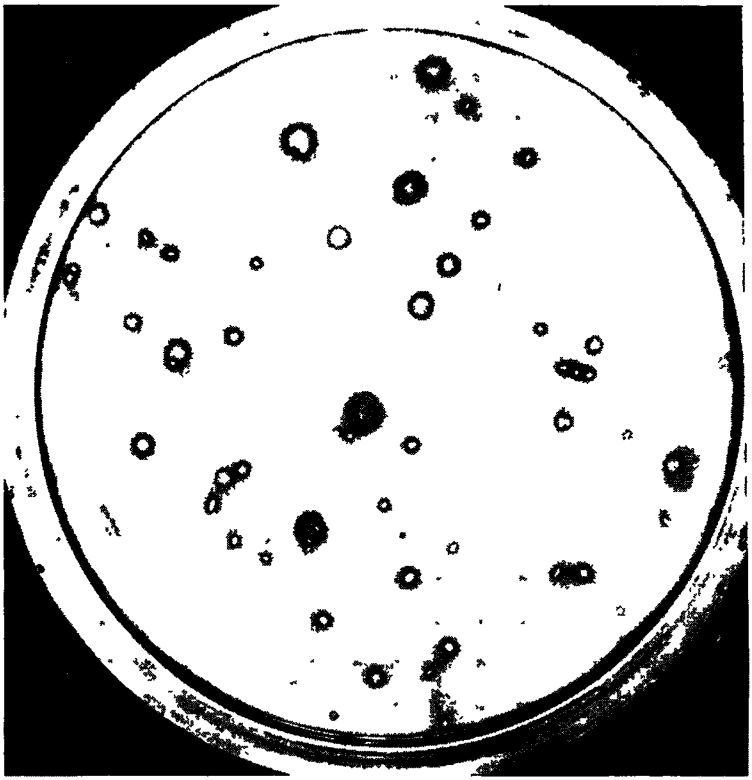


Figure 1. Sample plate of a dilution from a resin enriched soil showing bacterial colonies and their zones of halation or resin breakdown.

Isolation of Resin Attacking Bacteria By Enrichment Culture

The standard method of isolating pure cultures of bacteria is that of elective culture. This is effected by enrichment cultures in which a small amount of the substance under investigation is added to a large amount of soil and the admixture incubated. If the substance should not be too toxic in the concentration used, a microflora capable or metabolizing that particular substrate develops, when further addition of the substrate may be made. Finally, a small quantity of the bacterially enriched soil may be transferred to a soil-free mineral salt medium in which the substrate (in this case a resin) is the only organic foodstuff. If under these conditions bacteria multiply, then they are eminently capable of metabolising the substance concerned and, after repetitions of the above process, may be isolated by the technique (plating) of diluting the culture with a melted nutrient agar jelly and subculturing from one of the immobilized colonies that develop during cooling, solidification, and incubation.

Selective Counts by a Plating Technique

If the agar medium described above be so prepared that it contains only a resin as an available foodstuff (assuming agar to be almost nonnutritive, as it is to most terrestial organisms); then this can be used selectively to count the resin-attacking microbes present in soil and for their isolation and culture. Gum rosin and Vinsol are both insoluble materials and cannot be directly incorporated in a form sufficiently dispersed to be uniformly available to microörganisms. However, a fine emulsion in poured agar plates (as above) presents a much-greater surface for attack than larger aggregates.

acid nucleus. The sodium salts are then dispersed into the basal salt solution described below at a concentration approximately 0.2 percent, weight by volume at 90 C.

If, as was generally the case, the agar medium was being prepared, the agar was previously dissolved by boiling, since its subsequent peptising effect helped to ensure the stability of the resulting emulsion. The result was a stable milky-white opaque medium, the reaction of which was subse-

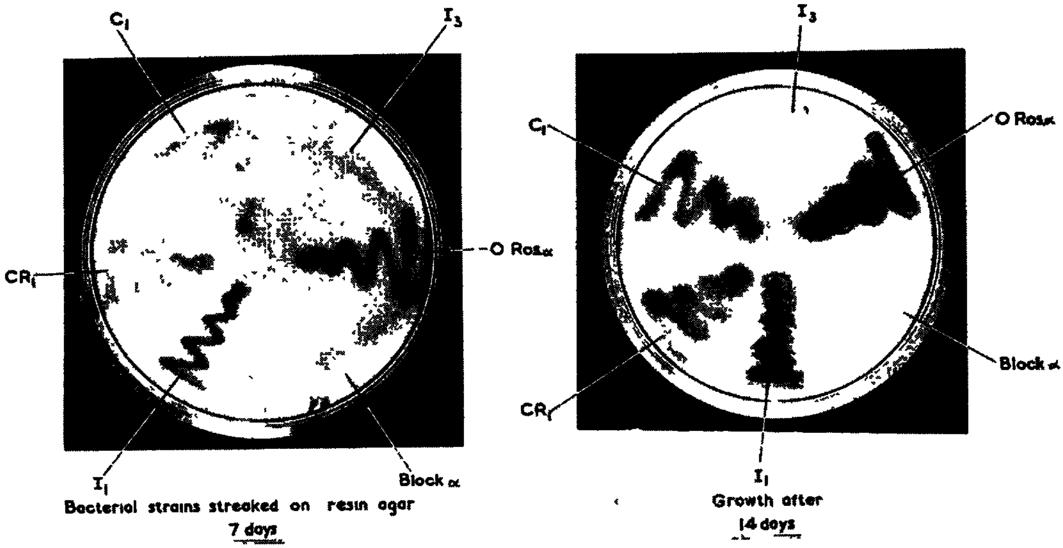


Figure 2. Pure culture streaks of resin attacking bacteria, showing halation and dissimilation of a colloidal resin agar.

In their studies on Guayule resins, Allen, Naghski, and Hoover (1) obtained a resin emulsion by dispersing an acetone solution of the resin into a mineral salt solution. In the opinion of the author, an emulsion is better obtained by prior saponification of the resin and dispersion into a buffer mineral-salt solution.

This is thought to be preferable, since in the former method, despite boiling to expel the acetone or ethanol, sufficient may still be present to affect the bacterial metabolism; the use of only nonorganic mineral solution precludes this.

The selective medium is prepared thusly: The resin is saponified with the calculated quantity of caustic soda, or, in the case of gum rosin, the commercially available sodium rosinate can be used. It is assumed that the relatively mild treatment of saponification will not affect the abietic

quently adjusted with normal NaOH to a pH of 7.0 - 7.2.

BASAL SALT SOLUTION

K_2HPO_4	0.35 gm.	
KH_2PO_4	0.15 gm.	
NaCl	0.10 gm.	
$MgSO_4 \cdot 7H_2O$	0.10 gm.	per litre
NH_4NO_3	0.10 gm.	
$CaSO_4$	0.05 gm.	
$FeCl_3$	Trace	

The agar emulsions thus obtained did not break on sterilization and could be stored. Since the dissimilation of the resin emulsion by the bacteria or fungi clarified the agar, the presence of a clear zone or halo around a bacterial or fungal colony on a resin-agar plate was indicative of attack, and the medium was thus suitable both for isolating and for counting resin-attacking organisms.

Examples of the use of this medium are seen in Figures 1, 2, and 3, which are respectively photographs of a count plate of resin attacking bacteria from soil, of pure culture streaks of resin-attacking bacteria, and of resin-attacking fungi. Other insoluble stabilizing agents were always investigated in a similar manner.

Methods for the Detection and Estimation of Resins

The classical test for substances having the menthadiene structure of an isopropyl unit attached to a ring structure with two conjugated double bonds is the Liebermann-Storch reaction (9), which is based on the color obtained when concentrated sulphuric acid or a saturated solution of $ZnCl_2$ in glacial acetic acid is added to a chloroform or acetic anhydride solution of these substances in the presence of an acyl- or aryl-chloride or anhydride (21).

This author has found the test given with benzoyl chloride and 40 percent of $ZnCl_2$ in glacial acetic acid to be fairly reliable when used either with experimental resin emulsions or with ether extracts of soils. However, the color obtained is fugitive, and unless techniques for accurate colorimetric estimation are available, it is better either to weight a simple ether extract, if the ether soluble solids of the natural soil are not too high, or to use the nitration method of Donath (25), which (although not so specific) gives permanent colors for comparison.

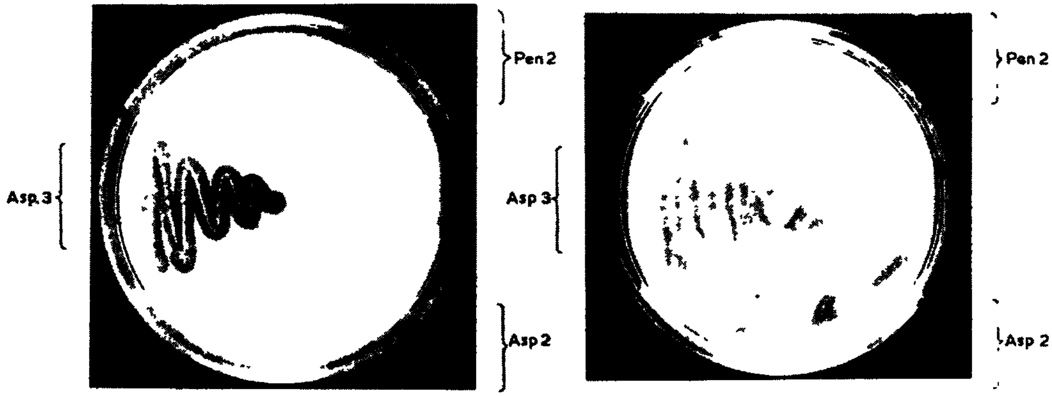
Methods for Studying Water Absorption

The methods adopted for studying water-absorption by resin-soil mixtures under various microbiological conditions were those used by the Road Research Laboratory.

Two different types of specimen were prepared. The majority were made in the laboratory compacting apparatus described by Little (22). In this method, 150-gram batches of moist soil-resin mixture are compacted into cylindrical specimens 2 inches in diameter and approximately $1\frac{1}{2}$ inches high and are allowed to cure in a moist atmosphere for 24 hours (i. e., to allow the spread of the resin film). The specimens are then removed, and the cylindrical surfaces coated with wax.

In the present investigations they were allowed to absorb water through their lower flat surface for varying periods of time, either by standing on saturated sand contained in a sieve or on the surface of a perforated metal sheet maintained $\frac{1}{2}$ inch below the surface of water in a closed metal tank.

Specimens containing less soil than the above were also used; they were cylinders 1 inch in diameter, containing about 30 grams of moist soil-resin mixture. These were compacted in a small steel mold of the appropriate dimensions by means of a plunger which was subjected to 8 blows of a $5\frac{1}{2}$-lb. hammer, (22) falling through a height of 1 foot. The specimens thus



Fungi inoculated on to colloidally dispersed resin agar 7 days

Development after 14 days

Figure 3. Pure culture streaks of resin attacking fungi, showing halation and dissimilation of a colloidal resin agar.

produced were about 1½ inches high and had a compacted soil density of the same order as that obtained in the standard compaction test. These specimens were cured and tested as described above.

RESULTS OF INVESTIGATIONS

Isolation of Resin-attacking Organisms

A large number of resin-attacking isolates were obtained from various sources, both by elective culture and by the use of the selecting-plating media described above. It was thought that the intestinal flora might contain some organisms that would attack sterols and the closely related abietic acid. This proved to be the case, and two very-active resin attackers were isolated from human faeces. All the bacteria isolated except those latter, show a morphological resemblance to the phenol, cresol, and naphthalene attackers isolated by Gray and Thornton (11). Moreover, two active resin-attacking fungi have been isolated, strains of Aspergillus nidulans and of Aspergillus versicolor. A proactinomycete was also isolated but was lost out of culture before it could be fully investigated. It is perhaps an unwarranted generalization to state that these organisms are of ubiquitous occurrence, but their presence has been demonstrated within a wide range of soils. These have comprised clayey soils at Rothamsted, sandy acid loams, and the calcareous brickearth from Harmondsworth (pH 8) that has frequently been used as an experimental material for this study. Even a heavily polluted London soil has given isolates of these organisms. However, no resinolytic organisms were demonstrated in marine muds.

The actual numbers of organisms present varied, and although no survey of relative numbers of organisms has been made, it would seem that it is in those soils in which a considerable zymogenous flora might be supposed to be present that the highest numbers of resin attackers were found. These comprised heavily manured farmyard and allotment soils, whereas a light chalky (calcareous) downland soil near Dover, Kent, proved to have a relatively small flora. Moreover, in a private communication, Yao Tsen Tchan of the Institut Pasteur, Paris, has also indicated that he has isolated a bacterium that appears to attack gum rosin.

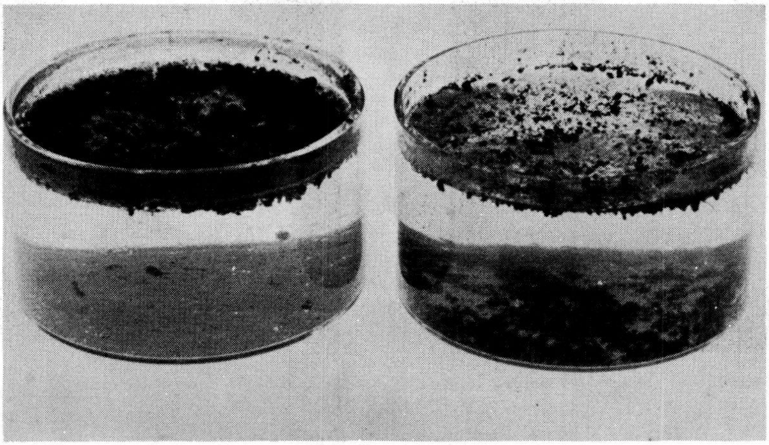


Figure 4. The effect of mercury in maintaining resinous soil stabilization (see Text).

Development of a Resinolytic Flora

The graphs shown in Figure 5 demonstrate the growth and increase in numbers of resin-attacking organisms, both in pure culture and in natural soil. (It is of interest to note the availability of blue copper rosinate in this context, which might a priori be supposed to be toxic.)

Demonstration that Increased Bacterial (and Fungal) Action Reduces the Effectiveness of Resins in Preventing Water Absorption

In general, bacterial counts made from samples of resin-stabilized roads showed an increase in resin-attacking organisms, particularly when failure was apparent. The introduction of 10 percent of cement in addition to the resin reduced bacterial numbers practically to extinction, but it is the opinion of the author that rather than using cement as a resin preservative in the proportion suggested (or slightly higher), it would be itself a treatment of choice.

LABORATORY EXPERIMENTS

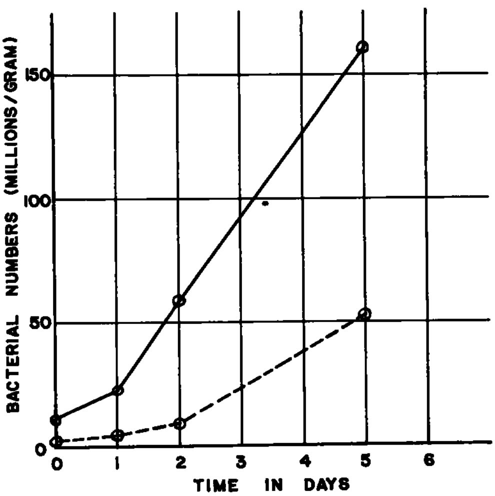
In some early experiments, the effect of alternate wetting and drying on small specimens was investigated. In this work, half the specimens were allowed to absorb water from a culture of known resin-attacking bacteria, and the others from a 0.2-percent solution of mercuric chloride, a vigorous sterilizing agent. In Figure 6 the effect of this wetting and drying can be seen; this effect was uniformly repeatable. Probably two factors are here present, both that of the mercuric ion in stabilizing the resin film, and its effectiveness as a sterilizing agent. (The bacterial numbers otherwise obtained from resin-enriched soils are greatly in excess of those from comparable rich natural field soils.)

In Table 2 are seen the results of another typical experiment in which 150-gram specimens of an unsterilized brick-earth (air dried) were stabilized with 1 percent of gum rosin at a moisture content of 12 percent and compacted in the Dietert compactor. Three sets of specimens were respectively uninoculated, inoculated with resin-attacking bacteria, and with resin attacking fungi. After curing and waxing, they were allowed to

TABLE 1

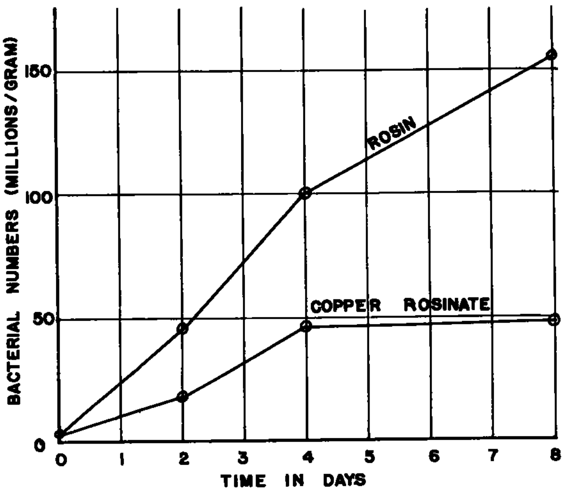
CHEMICAL AND PHYSICAL CHARACTERISTICS OF VINSOL RESIN AND ROSIN

	Vinsol resin	Gum rosin
Acid number	90-100	150-165
Methoxyl content	5-6%	Neg
Melting point (drop)	110-115°C	75-85°C
Noncarboxylic -OH (Zerewitnof)	6 %	Neg
Gasoline-soluble material	8-15%	90-100%
Approximate molecular weight	450	305
Combustion analysis (approx)		
Carbon	73%	79%
Hydrogen	7%	9.5%
Oxygen	20%	10.5%
Approximate formula	$C_{27}H_{30}O_6$	$C_{20}H_{30}O_2$



GROWTH OF RESIN-ATTACKING BACTERIA IN PURE CULTURE.

SUBSTRATE:
—— GUM ROSIN
---- "VINSOL"



DEVELOPMENT OF A RESINOLYTIC FLORA IN SOIL.

SUBSTRATES USED:
GUM ROSIN AND COPPER ROSINATE
MEDIUM USED:
SELECTIVE ROSIN MEDIUM

Figure 5.

TABLE 2

WEIGHT OF RESIN-STABILIZED SOIL SPECIMENS THAT HAD BEEN INOCULATED WITH RESINOLYTIC BACTERIA AND FUNGI

(Average weight of four specimens) (Standard errors given)

Time (Days)	Control	Inoculated with bacteria	Inoculated with fungi
1	149 0 ± 1 3	150 0 ± 1 5	149.5 ± 1 2
2	150 0 ± 1 4	151 5 ± 1 5	151 5 ± 1 4
3	151 0 ± 1 3	154 0 ± 1 5	154 0 ± 1 4
4	152 0 ± 1 3	156.0 ± 1 0	156 0 ± 1 6
5	152.5 ± 1 4	159 0 ± 1 6	159 0 ± 1 9
7	155 0 ± 1 2	164 5 ± 1 9	168 0 ± 2.0
8	156 0 ± 1.5	166 0 ± 1 9	171 0 ± 2 1

absorb water from a free water surface. It will be seen that the addition of such massive starters results in increased water uptake, presumably on account of the breakdown of the resin film. (Sterilized soil was not used, on account of the physical changes that probably take place during such pretreatment, although resin attack, per se, is easily demonstrable if such a soil be inoculated.)

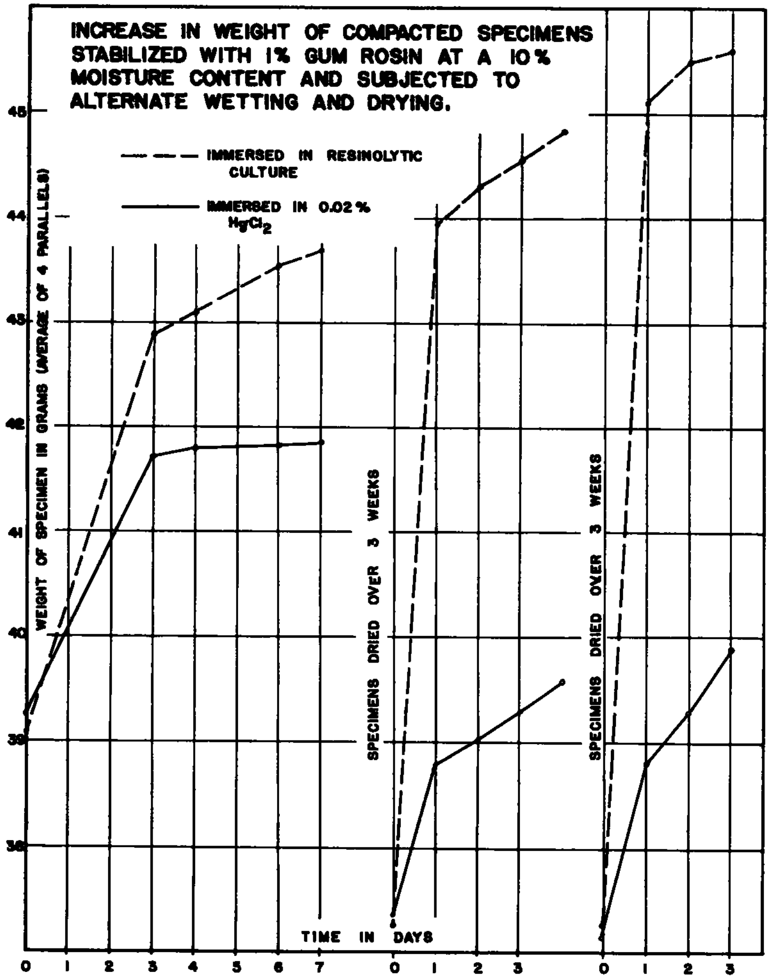


Figure 6.

TABLE 3

COUNTS MADE FROM SOILS THAT HAD BEEN INCUBATED (25°C) BOTH AEROBICALLY AND ANAEROBICALLY FOR FOURTEEN DAYS

Numbers of organisms (millions per gm.)

	Soil Alone	Soil + 1% Gum Rosin	Soil + 1% "Vinsol"
Aerobic	0.64	31.19	6 25
Anaerobic	---	18.90	0.63

These results suggest that the presence of resins in natural soils results in a great increase in the bacterial population of that soil and that the introduction of an extremely high resinolytic population into soil results in greatly impaired soil stabilization.

EFFECT OF PHYSICAL CONDITIONS AND OF ANTISEPTICS

The effect of physical conditions has been discussed above, and it is not proposed to deal with this subject at length here. However, a few results of interest will be mentioned. The effect of partial anaerobiosis (likely to be the case in the pore spaces of soil) was investigated by partially replacing the atmosphere of a dessiccator (used as a humidity chamber) with CO_2. The effect of such partial anaerobiosis is seen in Table 3, resin-attacking organisms being counted by means of the selective media described above.

An even-more-marked effect was seen with the two strains of resin-attacking fungi. Hence, as has readily been seen from other work, the resin-attacking organisms are aerobes, and their numbers can be reduced by lowering the oxygen tension[2]. From this observation it would immediately seem that the effect of compaction in reducing the pore space will reduce the available oxygen and thus (under active metabolic conditions, where microörganisms are respiring freely) a low oxygen tension will soon be achieved.

The results of Hubbell and Gardner (17) are of particular interest in this matter, since they observed a reduction in microbial numbers upon compaction in soils to which no addition had been made; while the results of the present author have tended to confirm this observation (though not without exception).

It would seem that further investigation of the effect of microenvironmental conditions might be of value in the conservation of resin stabilized foundations. Moreover, the observations made by the present author (26) and by others, such as Van Suchtelen (30), Engberding (8), and Jensen (19), have shown the dependence of bacterial numbers upon soil moisture content, although, as shown by the present author (Skinner, Jones and Mollison (26), there is a strong interaction with a temperature effect. Nevertheless when this is eliminated, the effect of soil moisture is still apparent. As may be expected, however, once no capillary pore space remains, a condition of waterlogging and of partial anaerobiosis occurs, and the bacterial numbers are reduced, according to Subrahmanyan, (33). Moreover, Hoffman (13), has suggested that the optimum conditions for soil bacteria occur when half the pore space is filled with water. (In the case of sandy soils, 8 to 10 percent; and with heavy clays 20 percent.)

The above observations may serve to give some indication of the mutually opposing factors that have to be resolved in this problem. For instance, to recapitulate, low moisture content upon compaction will tend to control the bacterial numbers within limits, but the effect of water ingress will be a cumulative one on the microflora up to the point of waterlogging, when the road foundation will not be useable in any case. Apart from the limited measure of control that can be achieved above, in the present work an investigation of the effect of antiseptics was made which will be briefly described below.

[2]Note by H. F W · Experiments performed at Princeton University on resin and asphalt stabilized lateritic soils showed strong microbial action in completely immersed specimens accompanied by reduction of iron from the trivalent to the bivalent form

EFFECT OF ANTISEPTICS[3]

It was early realized that for antiseptics to be a practicable proposition in preventing the breakdown of resins in soil, they must be cheap in the quantities that would have to be used and preferably inorganic in nature; since the ability of soil flora to metabolize any substance that affords a source of energy is amazing indeed. Moreover, unless the substance is strongly surface active, it will be difficult to achieve a toxic concentration throughout the soil at an economic cost.

The attentions of the present author turned therefore to a selection of antiseptics as follows; sodium pentachlorophenate, bleaching powder, (calcium hypochlorite) copper sulphate, mercuric chloride (corrosive sublimate) and many others. The effect of soil and of resin pretreatment was investigated to some extent.

Laboratory experiments with media of colloidally dispersed copper rosinate and mercuric resinate, made by adding the calculated quantity of $Cu^{\cdot\cdot}$ or $Hg^{\cdot\cdot}$ to the basal salt solution in preparing the select-

[3]Note by the Editor See also "Fundamental Approach to the Stabilization of Cohesive Soils" by Hans F Winterkorn Proc 28th Annual Meeting, Highway Research Board, pp. 415-422 (1948)

TABLE 4

BACTERIAL NUMBERS OBTAINED BY PLATING SOILS INCUBATED FOR 10 DAYS AT 25°C - ADDITIONS AS IN TEXT

Specimen	Average No of organisms (Millions per gram)	
	Nutrient	Resin
1% Gum Rosin + inoculum	332 69	283 75
Inoculum alone	97 87	23 87
1% Gum Rosin + inoculum + 1% CuSO⁴	116 12	90 56
1 % Gum Rosin + inoculum + 0 02% $HgCl_2$	0 31	0 19
1% Gum Rosin + inoculum + 0 02% sodium pentachlorophenate	0 37	0 12

ive media as above, showed that aspergillus nidulans and aspergillus versicolor would grow readily on a medium of blue copper rosinate, that bacterial attack was slightly reduced, and that neither would grow on the mercuric-rosinate medium. However, it has been noted above that an increase in bacterial numbers occurs on the addition to soil of copper rosinate. It therefore seemed that copper would be of little avail in preventing resin decomposition, despite its known antimicrobial use (in wood preservation and against powdery mildews for instance.)

These results are apparent in Table 4, in which a number of loose specimens of sterile Harmondsworth sandy loam were made up to a moisture content of 10 percent, a resin content of 1 percent, and a standard inoculum of resin-attacking organisms added. It will be noted that sodium pentachlorophenate was also effective in reducing bacterial numbers. The results of a similar experiment with gum rosin and Vinsol are shown in Table 5, where with a moisture content of 12.5 percent similar results are seen.

Some further tests were then made on the influence of the antiseptic of the stabilizing agent. Two series of compacted specimens of 2.5 cm. diameter were made from the same soils with eight blows of a 5½-lb. hammer. In the first series (A) the specimens were made concurrently with the bacterial count, and in the second (B) the soil was stored over a period of six weeks and then rewetted to 7.5 percent moisture content. All the specimens were then allowed to absorb water on a wet sand surface for 4 days; the final results of this experiment are given in Table 6.

It will be noted that a significant effect of $HgCl_2$ is only evidenced in Series B, where loose samples had been incubated. In all cases, adequate opportunity for the establishment of a resinolytic flora was present (the soils were inoculated), so that compacted specimens showed less tendency to break down than those stored loose. This is consistent with the hypothesis that the resinolytic organisms are aerobic. It may also be noted that too high a proportion of sodium pentachlorophenate affects the waterproofing of the resin used, but a low concentration reduces the number of resin attackers. However, it is known that chlorophenols in low concentration (as might be expected) are decomposed in soils (38) and thus any ameliorative effect is likely to be short lived).

Again, in the search for economically suitable antiseptics, bleach (calcium hypochlorite) was used. The results of a test using this, $HgCl_2$, and sodium penthachlorophenate are given in Table 7. It will be seen that calcium hypochlorite is useless, despite its antiseptic activity, since stabilization is impaired by the antiseptic agent.

An attempt, moreover, was made to effect antisepsis by chlorinating the resin molecule (the resins readily absorbed wet chlorine, with a rapid exothermic reaction); however, once again, the introduction of chlorine into the abietic acid molecule (presumably, by saturation of

TABLE 5

Specimen	Count on nutrient agar (millions/gm)	Count on rosin agar (millions/gm)	Count on "Vinsol" agar (millions/gm)
12 5% moisture + 1% Gum rosin + inoculum	267 76 (spreading present)	(412 20) insufficient plates	-
"	431 04	412 40	-
12 5% moisture + 1% Gum rosin + 0 1% S P C P + inoculum	0 32	nil	-
12 5% moisture + 1% Gum Rosin + 0 78% $HgCl_2$ + inoculum	0 32	nil	-
as above but with 0 25% and 0 5% S P C P	no colonies whatever		
12 5% moisture + 1% "Vinsol" + inoculum	538 88	-	122 88
ditto	429 44	-	123 60
ditto	503 04	-	101 20
12 5% moisture + 1% "Vinsol" + 0 1% S P C P + inoculum	137 32	-	nil
12 5% moisture + 1% "Vinsol" + 0 078% $HgCl_2$ + inoculum	2 28	-	nil

the conjugated double bonds) reduces the hydrophobic nature of the anionic group and thus defeats the intended purpose.

However, a different story always held with mercuric chloride, which was invariably effective, even in low concentrations. However, the high price and high toxicity of this product would seem to invalidate its use in the field. However, it would seem that some hope can be offered should Hg^{++} be so incorporated in the resin as to reduce the toxicity of the product to the handler, if the cost were still economic and the surface properties of spread and stabilization unaffected, then this alone would seem to offer any hope for the future.

EFFECT OF SOIL PRETREATMENTS

Various methods of field soil sterilization were tried in this context (e.g., steaming, formalin-and-sulphuric-acid treatment, and combinations of these). With the exception of sulphuric-acid treatment (which was most probably a pH effect (Final pH 4) none were efficaceous, and indeed gave the surprising result of inducing a vigorous resinolytic flora to such a marked extent that the recent use of formalin in a resin-free soil could readily be traced by observing the increase in resin-attacking organisms by plating on the selective media described.

TABLE 6

WATER UPTAKES OF SPECIMENS TREATED WITH VARIOUS ANTISEPTICS

(Mean weight of four specimens)
(Significant effects indicated with asterisks)

Treatment	Series A (compacted)			Series B Incubated loose (then compacted)		
	Air Dry Wt	Final Water Uptake	Percent Water Uptake	Air Dry Wt	Final Water Uptake	Percent Water Uptake
1% Rosin	23 80	2 13	8 95	42 24	6 37	15 08
" + $HgCl_2$ 0 078%	22 23	1 92	8 64	39.93	3 21	8.04
" + SPCP 0 1%	23 28	1 87	8 03	42 95	2 80	6 66
" + SPCP 0 25%	23 68	2 72	11 48	39 80	5 13	15.40
" + SPCP 0 5%	23 51	5 19	22.08	40 75	6 61	16 21
1% "Vinsol"	24 07	1 55	6 44	35 13	3 65	10 39
" + $NgCl_2$ 0 078%	24 33	1 37	5 63	36 50	2 99	7 77
" + SPCP 0 1%	24 64	2 03	8 24	39 86	3 32	8 31
" + SPCP 0 25%	22 60	3 04	13 45	40 24	4 88	12 13
" + SPCP 0 5%	23 95	4 11	16.77	41 11	7 50	18 46

TABLE 7

EXPERIMENT ON A SANDY LOAM SOIL
Schedule of different treatments

(S) Soil alone
(V) Soil + 1% "Vinsol"
(VP) Soil + 1% "Vinsol" + 0 1% Na pentachlorophenate
(VH) Soil + 1% "Vinsol" + 1% Calcium hypochlorite
(3) Soil + 1% "321" resin
(3H) Soil + 1% "321" resin + 1% Ca hypochlorite
(3M) Soil + 1% "321" resin + 0.016% $HgCl_2$

TABLE OF RESULTS

(Initial weight of soil - 150 gms)
(moisture content 10%)
(Average of 2 specimens only, but 5% variation was maximal)

	Weight after drying (gms)	Weight after 1 week (gms)	Total water Uptake (gms)	Percentage Uptake
S	129 2	171 2	42 0	32 5
V	130 2	138 4	8 2	6 3
VP	131 2	136 4	5 2	4 0
VH	132 2	168 1	35 9	27.3
3	129 0	133 6	4 5	3 4
3H	132 1	141 4	9 3	7 1
3M	132 8	134 9	2 1	1 6

In some other experiments the effect of Ca hypochlorite invariably was to render the specimen unmanageable

It would therefore seem that these organisms are an integral part of the normal soil balance and that resin decomposition is only one of their talents. Thus, from the above investigation, admittedly very limited, it would seem that within the small range of antiseptics tested, mercury alone presented any advantage. How striking this may be is demonstrated by the following simple final experiment.

Two similar specimens, both stabilized with Resin 321 and one containing 0.016 percent of $HgCl_2$, were prepared in the Dietert compactor at a moisture content of 10 percent. After incubation for two weeks, both were dried in an oven and then powdered. Five grams from each were then taken and spread uniformly over a distilled-water surface in a 4-inch crystallizing dish and left overnight. At the end of 12 hours, the surface soil was agitated in both cases; the soil from the specimen that contained no mercury wetted at once (A), whereas that from the specimen that had contained mercury did not wet at all.

This experiment is illustrated in Figure 4. The effect is probably dual: that of the mercury in stabilizing the resin film, and its effect in preventing microbial attack. (Some of this work forms the subject of a patent in the author's name invested in the Imperial Trust.)

DISCUSSION

From the above, it is apparent that when complex decomposable substances are added to natural soils, unless they have a very-unusual structure or are present in high concentration, then their decomposition over a period of greater or less time by the soil microflora is virtually assured. However, this unfortunate fact must always be viewed from an economic point of view. If the useful life of a resin-stabilized foundation, with or without antiseptic, is suited to the economics of the situation (such as a temporary road as a precursor to some more-permanent foundation) then it has everything to recommend it. Moreover, it has the advantage that temporary disturbance is easily repaired (one reason for wartime development).

However, for longer term planning, the relative cost of more-permanent means of consolidating a foundation should be considered at the present state of knowledge, where the cost of an adequate quantity of a suitable stabilizing or binding agent should always be a primary factor. But a more-vigorous attack on the problem of resin decomposition would probably pay dividends, particularly where road construction in areas away from suitable stone is considered.

If the microbiological problem can be solved, then immense areas of lateritic soils in the tropics may well be opened to trade by road stabilization using the native resins. The produce thus freed to a hungry world would aid the development of such backward areas and pay for more-permanent roads to provide the means of permanent contact with the outside world, the spread of civilization, and the defeat of that bogey of the near future: world famine.

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