HIGHWAY RESEARCH BOARD
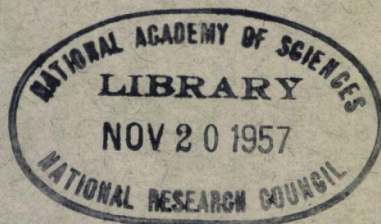## Bulletin 118

Effects of Chlorination and Microorganisms and Constituents of Asphalts

National Academy of Sciences—
National Research Council

publication 405

HIGHWAY RESEARCH BOARD

Officers and Members of the Executive Committee
1955

HIGHWAY RESEARCH BOARD
Bulletin 118

Effects of Chlorination and Microorganisms and Constituents of Asphalts

PRESENTED AT THE
Thirty-Fourth Annual Meeting
January 11–14, 1955

1956

Washington, D. C.

Contents

Effect of Chlorination on Oxidizability of Road Asphalts

FREDERICK C. SANDERSON, Assistant Professor of Civil Engineering
Yale University

Oxidation of the asphaltic binder in bituminous pavements is a frequent cause of pavement failure. The hardening, or loss of penetration of the asphalt which results from oxidation occurs gradually over the life span of the pavement. It can also take place fairly rapidly at the elevated temperatures used during mixing operations.

This paper describes some of the physical and chemical effects of direct chlorination on road building asphalts. In the laboratory, experimental data have been obtained which indicate that the oxidizability of the asphalt may be materially reduced by treatment with chlorine.

The action of chlorine on asphalt is in many respects similar to that of oxygen. However, it is to be expected that the loss of penetration resulting from treatment with limited amounts of chlorine will be less than that resulting from a chemically comparable amount of oxidation. Because of the fact that chlorine is in general more active chemically than oxygen, it is also to be expected that the compounds formed in asphalt by treatment with chlorine will be relatively stable and that the resistance of the asphalt to oxidation will be increased.

In the laboratory, eight samples of asphalt cement were subjected to direct chlorination. Tests performed on the samples before and after chlorination, including the Anderson-Stross-Ellings tests, indicate that although the penetration of the asphalt cements tested is decreased by chlorination, the resistance to hardening is greatly increased.

● FAILURES of asphalt pavements other than those resulting from poor subgrade conditions or faulty pavement design appear to be due primarily to one of two causes; stripping, or hardening of the asphalt.

Loss of penetration or hardening occurs gradually by weathering over the life span of the pavement. It can also take place fairly rapidly at the elevated temperatures used during mixing operations. In the case of asphalts containing appreciable amounts of volatile materials, the drop in penetration may be caused by loss of these materials through evaporation. In the case of most asphalts, however, it has been shown that the hardening is caused by oxidation of certain constituents of the asphalt.

There are many factors which influence the occurrence and rate of failure of a bituminous pavement by gradual oxidation or weathering; among these being the susceptibility of the asphalt to oxidation, the average temperature to which the pavement is exposed, the density of the pavement, and the amount of asphaltic binder present in the mix. Since the oxidation of asphaltic materials is catalyzed by heat and by actinic light rays, oxidation begins on the surface of the pavement and progresses gradually downward. The surface becomes brittle, cracks appear which permit the entrance of air and moisture, and eventually the pavement fails.

Because of the high temperature (300 to 350 F.) used in mixing sheet asphalt or bituminous concrete made with asphalt cement, oxidation can occur very rapidly and there may be a significant drop in penetration before the mix is even in place. In this case, the expected life span of the pavement is materially shortened.

It is the purpose of this report to describe some of the physical effects of direct chlorination on road building asphalts, and to present experimental data suggesting that the loss of penetration resulting from oxidation may be materially reduced by treatment with chlorine.

THEORETICAL BACKGROUND

Asphalt, either natural or manufactured, is a mixture of various organic compounds which have been arbitrarily classified as: (1) carboids, that fraction of the asphalt which
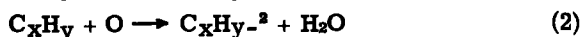
1

is insoluble in carbon disulfide; (2) carbenes, the fraction which is insoluble in carbon tetrachloride but soluble in carbon disulfide; (3) asphaltenes, which are soluble in carbon tetrachloride but insoluble in low boiling saturated hydrocarbons; and (4) maltenes, which are soluble in low boiling saturated hydrocarbons such as pentant (12). These constituents exist in asphalt in varying proportions as a colloidal mixture, the asphaltenes making up the major portion of the disperse phase, while the intermicellular phase consists primarily of the maltenes. Except in highly cracked or oxidized asphalts, the percentage of carbenes and carboids is very low.

About 35 to 60 percent of the compounds in the maltene group are saturated paraffinic compounds. From 10 to 30 percent are alicyclic compounds of the cyclohexane type, and the balance of the maltene fraction is made up of aromatic compounds either in aromatic structure or in various combinations with the paraffins and alicyclic compounds.

Little is known concerning the true nature of the asphaltenes. Analysis has shown, however, that they can be considered as "consisting of aromatic hydrocarbons containing paraffin chains to a varying extent, depending on the origin and manufacture of the bitumen" (12).

Oxidation of an asphalt at either high or low temperature results in a brittle, gel-type colloid if the oxidation is carried far enough. According to Abraham:

> The effect of oxidation is two-fold, and involves the direct union of oxygen with the bituminous substances, also the elimination of a portion of the hydrogen or carbon in the form of water or CO_2. These reactions may be expressed roughly as follows:
>
> $$C_xH_y + O \rightarrow C_xH_yO \qquad (1)$$
>
> $$C_xH_y + O \rightarrow C_xH_{y-2} + H_2O \qquad (2)$$
>
> also
>
> $$C_xH_y + 2O \rightarrow C_{x-1}H_y + CO_2 \qquad (3)$$
>
> (1, p. 1464)
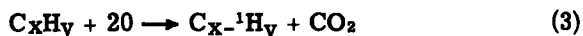
A reaction of Type 2 requires a greater amount of activating energy such as heat or actinic light rays than a reaction such as that indicated by Equation 1, while a reaction of the type shown in Equation 3 requires still further amounts of energy to progress in the direction indicated. It is highly improbable that oxidation of Type 3 could occur even at the elevated temperatures used at the mixing plant.

From the standpoint of molecular structure, oxidation without doubt results in the formation of larger molecules through reactions closely akin to polymerization and co-condensation. In the case of oxidation by simple addition, cross-linkages of an "ether bridge" type may be formed:

```
              H                  H
              |                  |
 H - C = C - C - X + H - C = C - C - X + O ⟶
     |   |                  |   |
     H   H              H   H


     H   H
     |   |
 H - C - C - C - X
     |       |
     |       H
     O
     |       H
     |       |
 H - C - C - C - X
     |   |   |
     H   H   H
```
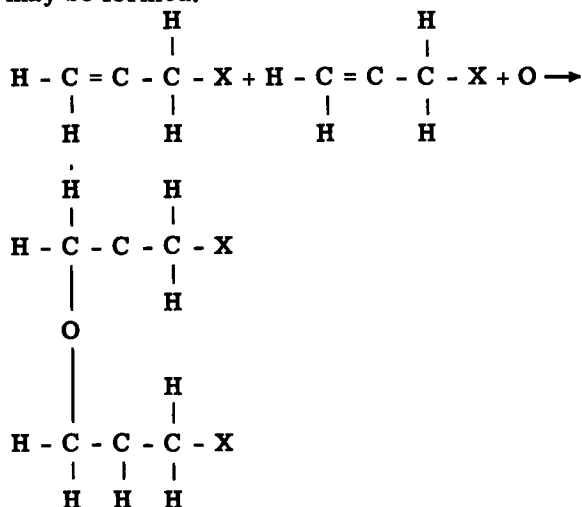
In the case of oxidation of the second type, cross-linkages may be formed with the elimination of hydrogen as water:

$$
\begin{array}{cc}
\text{H} & \text{H} \\
| & | \\
\text{H - C - C - X} & + \\
| & | \\
\text{H} & \text{H}
\end{array}
\quad
\begin{array}{cc}
\text{H} & \text{H} \\
| & | \\
\text{H - C - C - X} & + \text{O}_2 \longrightarrow \\
| & | \\
\text{H} & \text{H}
\end{array}
$$

$$
\begin{array}{cc}
\text{H} & \text{H} \\
| & | \\
\text{H - C - C - X} \\
| \\
\text{H} \\
| \\
\text{O} \qquad\qquad + \text{H}_2\text{O} \\
| \qquad \text{H} \\
| \quad | \\
\text{H - C - C - X} \\
| \quad | \\
\text{H} \quad \text{H}
\end{array}
$$
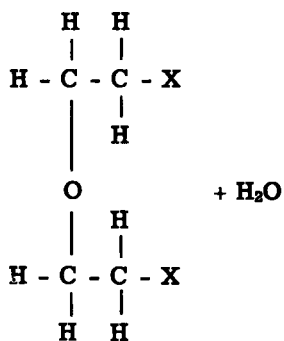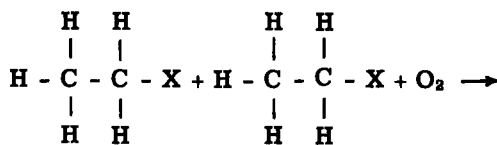
Or a sort of polymerization may take place with elimination of hydrogen and no addition of oxygen:

$$
4\left[
\begin{array}{cc}
\text{H} & \text{H} \\
| & | \\
\text{H - C - C - X} \\
| & | \\
\text{H} & \text{H}
\end{array}
\right] + \text{O}_2 \longrightarrow
$$

$$
2\left[
\begin{array}{cccc}
\text{H} & \text{H} & \text{H} & \text{H} \\
| & | & | & | \\
\text{X - C - C - C - C - X} \\
| & | & | & | \\
\text{H} & \text{H} & \text{H} & \text{H}
\end{array}
\right] + 2\text{H}_2\text{O}
$$
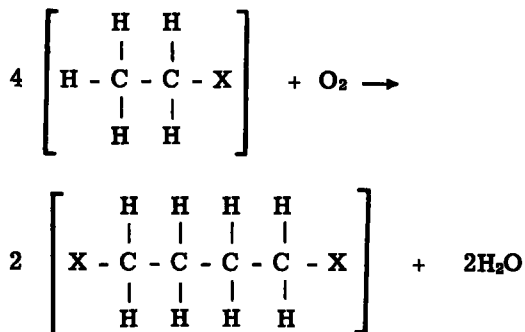
In any case, these reactions tend to result in a gel structure of the formation of cross-linked "chain" type molecules. This results at first in an increase in elastic properties of the asphalt, and finally in brittleness as the reaction proceeds further and further.

Although Pfieffer speaks repeatedly of asphaltene formation as a result of oxidation, there is no indication that compounds are formed which resemble the asphaltenes chemically. During oxidation, as has been shown above, compounds are formed which have larger molecules and a more complex molecular structure. These compounds are less soluble than the original compounds in low boiling hydrocarbons such as pentane, they have higher melting points, and in most cases they would tend to be more brittle and less ductile. It is therefore to be expected that an oxidized asphalt would have a lower penetration, a higher R and B temperature and that it would be less ductile than the original material.

The action of chlorine on asphalt is in many respects similar to the action of oxygen. If any compounds are present in the asphalt with olefinic double bonds, the reaction will be one of simple addition:

$$
\begin{array}{c}
\text{Cl} \quad \text{Cl} \\
| \quad | \\
\text{H - C = C - X} + \text{Cl}_2 \longrightarrow \text{H - C - C - X} \\
| \quad | \qquad\qquad\qquad | \quad | \\
\text{H} \quad \text{H} \qquad\qquad\qquad \text{H} \quad \text{H}
\end{array}
$$
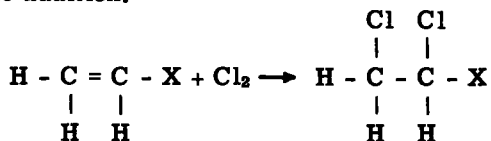
No cross-linkages will be formed, since chlorine is monovalent. Chlorine may also react with the hydrocarbons with the elimination of hydrogen in the form of hydrochloric acid:

```
    H   H                              Cl  Cl
    |   |                              |   |
H - C - C - X + 2 Cl₂          H - C - C - X + 2 HCl
    |   |                              |   |
    H   H                              H   H
```

or

```
      ⎡   H   H   ⎤                H   H   H   H
      ⎢   |   |   ⎥                |   |   |   |
   2  ⎢ H-C - C-X ⎥ + Cl₂      X - C - C - C - C - X + 2 HCl
      ⎢   |   |   ⎥                |   |   |   |
      ⎣   H   H   ⎦                H   H   H   H
```
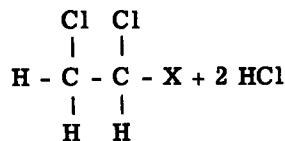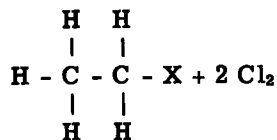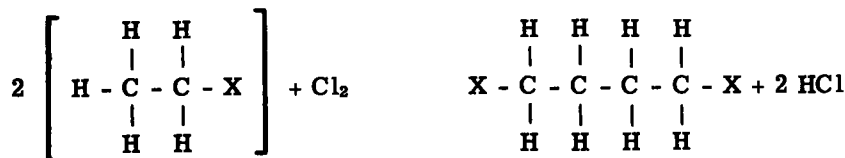
As in the case of oxidation, the simple addition reaction will take place more rapidly at lower temperatures than the reactions involving the elimination of hydrogen.

The idea of chlorinating asphalt is not new, and some of the properties of asphalt treated with comparatively large amounts of chlorine have been investigated.

Reaction with chlorine is an alternative technique by which asphaltic bitumen possessing certain special properties can be produced.

The action of chlorine is like that of sulfur and oxygen, i. e. chlorine withdraws hydrogen. The greater part of the added chlorine escapes in the form of hydrochloric acid.

Just as with sulfur, this reaction takes place at a lower temperature than is the case with oxygen, proceeding with ease at 200 C.... the action of the chlorine (compared with that of oxygen) on a Venezuelan asphaltic bitumen is shown in the following table.

The considerable analogy between the action of chlorine and that of oxygen is evident. A little more chlorine is, however, combined in the bitumen (12, p. 146).

TABLE 1

Treatment of Venezuelan Bitumen with Chlorine

(base stock: pen. at 25 C. 178, temp. R&B 39. 5 C.) 60/80 asphaltenes 12. 3%

Experimental Conditions	Treatment with Chlorine	Blowing with air
Temperature, deg. Centigrade	200	250
Consumption of agent		
per kilogram of asphalt	78g. Cl₂	21g. O₂
Volatile reaction products formed	73g. HCl	abt. 22. 5g. H₂O
Hydrogen withdrawn from asphalt	2g.	2. 5g
Agent absorbed (calculated)	7g. Cl₂	abt. 1 g. O₂
Properties of final products:		
Pen. at 25 C.	26	22
Temp. R&B	82	84
60/80 asphaltenes, %	26. 3	28. 4

(12. p. 147)

It can be seen from this table that both chlorination and oxidation of asphalt result in a decrease in penetration, an increase in R & B temperature, and an increase in the "asphaltene" content (percentage of the asphalt insoluble in a mixture of paraffinic hydrocarbons with a boiling range between 60-80 C.).

The change in physical properties which occur as a result of causing 78 grams of chlorine to react with 1 kg. of asphalt are practically identical with the changes which occur as a result of the action of 21 grams of oxygen on the same asphalt. If, molecule for molecule, the action of chlorine were identical with that of oxygen, it would be reasonable to expect that much less than 78 grams of chlorine would be required to produce this result. However, as has been explained previously, the "ether bridge," or cross-

linking type of reaction does not occur with chlorination. Therefore it is to be expected that the average molecular weight of the compounds formed by chlorination will be lower than that of the compounds formed by oxidation. If a sample of asphalt is treated with a given molecular quantity of oxygen, and another sample of equal weight is treated with the same molecular quantity of chlorine, the physical properties of the sample subjected to chlorination will change in the same direction as those of the oxidized sample, but to a much smaller extent.

When an asphalt is subjected to direct chlorination, the first reaction which takes place is one of simple addition at olefinic double bonds. These compounds will not be as easily oxidized as the unchlorinated compounds since they are now "saturated." As chlorination progresses, hydrogen will be displaced by chlorine at other reactive points in the molecules. Although some polymerization will take place, the loss in penetration and ductility of the asphalt will not be as great as if a comparable amount of oxidation had occurred. Furthermore, future oxidation will be retarded, since oxidation cannot take place at those points in the molecule which have already reacted with chlorine.

LABORATORY PROCEDURES

Eight samples of paving asphalt obtained from various manufacturers were tested in the laboratory. Of these samples, four were subjected to chlorination to determine the change in physical properties, any increase or decrease in resistance to oxidation, etc. Data furnished by the manufacturers is as follows:

Asphalt A. 100/120 penetration paving asphalt.

Asphalt B. 20/30 penetration pressure still tar produced as a result of cracking operations. M. P. 120-130 F.

Asphalt C. Flash point (deg. Fahrenheit)-575; penetration, 77 F. -67; ductility, 77 F. -150; ductility, 60 F. -150; sp. gr. , 77 F. -1.019; sol. CCl₄, %-99.7; oliensis spot test-negative.

After Shattuck oxidation test: penetration, 77 F. -25; ductility, 77 F. -150.

Asphalt D. Flash point (deg. Fahrenheit)-585; penetration, 77 F. -66; ductility, 77 F. 150; ductility, 60 F. -150; sp. gr. ,77 F. -1.1016; sol. CCl₄, %-99.8; oliensis spot test-negative.

After Shattuck oxidation test: penetration, 77 F. -45; ductility, 77 F. -150.

Asphalt E. 85/100 penetration paving asphalt from West Texas crude.

Asphalt F. 85/100 penetration paving asphalt from Near East crude.

Asphalt G. 85/100 penetration paving asphalt from Kansas crude.

Asphalt H. 85/100 penetration paving asphalt from Wyoming crude.

Asphalts E, F, G, and H were products of the same company, and asphalts E and F were produced at the same refinery. According to the manufacturer, asphalt B "is a pressure still tar which by nature of having been produced as a result of cracking operations contains a high percentage of unsaturated hydrocarbons."

Chlorination

Samples of each asphalt under consideration were treated with two different quantities of chlorine and tested to measure the change in physical properties, resistance to oxidation, etc.

Chlorination was carried out in the laboratory by "blowing" the asphalt with dry chlorine at a temperature of 150 C. This comparatively low temperature was chosen to favor simple addition reactions over those involving substitution. Chlorine was "blown" through 500-gram samples of asphalt with constant agitation at a rate of 100 cc. per minute. The reactions were carried out in 1 liter Erlenmeyer flasks.

In an attempt to determine the degree of chlorination which would produce the best overall results, each asphalt was chlorinated to the point where hydrogen chloride was evolved at the reaction temperature. The evolution of hydrogen chloride was detected by passing the gases emerging from the reaction flask first over dry mercury, and then into a beaker containing a solution of silver nitrate. Any chlorine present in the mixture of gases reacted with the mercury to form mercuric chloride. The presence of hydrogen chloride in the mixture was detected by a dense white precipitate of silver chloride

in the silver nitrate solution. After chlorination, the asphalt was heated to 200 C. for a period of 30 minutes to drive off any remaining hydrogen chloride.

The change in physical properties of the asphalt (i. e. loss in ductility and penetration) resulting from this first attempt at chlorination was so great as to offset any advantage gained in reducing the oxidizability. Therefore, a smaller amount of chlorine was used in treating the second series of samples.

Because of time limitations, tests were not run on chlorinated samples of asphalts E, F, G, and H. Except for a slightly higher penetration, the properties of E, F, G, and H resembled those of C and D.

Tests on Physical Properties

Specifications for determining the R & B softening point, the ductility, and the penetration permit a cooling time in air of only $\frac{1}{2}$ to 2 hours before immersion of the specimen in a water bath prior to the test. Pfeiffer has shown (12, pp. 157-160) that these properties may change considerably if the cooling time in air is increased or decreased only very slightly. Therefore specimens used in performing these tests were allowed to stand at room temperature for five days before immersion in the water bath. After a five-day "curing" period any change in properties which might result from allowing the specimens to remain in air at room temperature for an additional few hours would be negligible (12, p. 159).

Softening Point, Ring and Ball (AASHO T 53-42). Except for the five-day curing period mentioned above, this test was performed exactly in accordance with the specifications. The results are summarized in Table 2.

Ductility (AASHO T 51-44). Ductility tests were carried out at a temperature of 25 C. (77 F.). Except for the five-day curing period, the tests were carried out in accordance with specifications.

Penetration (AASHO T 49-44). The penetration of each sample was obtained at 0 C. (32 F.) with a 200-gram weight, penetration time 60 seconds; at 25 C. (77 F.) with a 100-gram weight, penetration time 5 seconds; and where possible at 46.1 C. (115 F.) with a 50-gram weight and a penetration time of 5 seconds. Except for the five-day curing period, the tests were performed in accordance with the specifications.

Determination of the Percentage of Bitumen Soluble in Carbon Disulfide (AASHO T 58-37). This test was performed in accordance with the psecifications.

Percentage of Bitumen Insoluble in Paraffin Naphtha (AASHO T 46-35). This test was performed in accordance with the specifications, except that pentane was used as the solvent rather than the higher boiling fraction specified.

Tests of Susceptibility to Oxidation

All of the proposed tests for oxidizability of road asphalts suffer from one or both of two defects. Most of the tests are carried out at temperatures well above the maximum temperatures found in bituminous pavements in place. Under these circumstances, oxidation will be accelerated, which is desirable, but as explained in the preceding pages the percentage of substitution reactions (i. e. elimination of hydrogen as water) will be higher than it would be if the test were carried out at lower temperatures. These tests can be used to predict oxidation during mixing operations, but they may not correlate well with performance of an asphalt pavement subject to oxidation by weathering.

The second defect found in many of the tests is that at some point during the test the asphalt is extracted or dissolved in an organic solvent such as benzene or carbon tetrachloride. The solvent is distilled off, and standard tests are run on the residue. Although it is possible to prevent further oxidation during distillation by distilling under carbon dioxide, it is often not possible to get rid of all the solvent. Obviously a very small amount of solvent remaining in the residue will drastically alter the physical properties.

The well-known Shattuck test is a case in point. This test suffers from both the above-mentioned defects.

In 1942, A. P. Anderson, F. H. Stross, and A. Ellings of the Shell Development Company proposed two tests for predicting the susceptibility of an asphalt to oxidation. The

first of these tests is simple in nature and involves nothing more than measuring the loss of penetration on heating. The second test proposed by these authors involves oxidation of the asphalt in a bomb with gaseous oxygen under pressure. This test is particularly suitable for predicting oxidation under weathering conditions since the temperatures involved do not exceed 50 C.

According to the authors, a standard loss of penetration on heating test does not correlate very well with behavior of the asphalt in service because the original penetration of the asphalt is not taken into consideration. The loss of penetration on heating is expressed as a percentage of the original penetration.

> A number of investigators have established the fact that when penetration of an asphalt drops below a certain point, the road in which the asphalt is incorporated will fail because of brittleness. The obvious conclusion, which also has been generally accepted in practice, is that a softer asphalt has the advantage over a harder asphalt of similar hardening characteristics, since the former will take longer to reach a critical penetration (5, p. 45).

The authors have developed the following method which takes into account the original penetration of the asphalt:

> The asphalt specimen is heated, in the conventional manner, to 16 C. for 5 hours in a standard oven after which the penetration of the sample is taken; this process is then repeated. The logarithms of the penetrations after 5 and after 10 hours are plotted against time in hours. A straight line is drawn through the two points and extended to 10 penetration, which is used as the x axis. The intercept on the x axis gives some indication of the time required by the asphalt to drop to this penetration and is termed the resistance to hardening.

$$\text{Resistance to hardening} \qquad 5\left[\frac{y_1-1}{y_1-y_2}\right] + 5$$

y_1 log penetration after 5 hours' heating
y_2 log penetration after 10 hours' heating
(5, p. 46)

In order to correlate the results of this test and those of the oxygen bomb test with the performance of the asphalt in a pavement the authors use a service rating.

> The service rating is a measure of the performance of an asphalt, and is derived from experimental paving sections in which the asphalts were used under mixing and laying conditions which were as well standardized as possible. These sections were strips of pavement laid in the San Francisco Bay Area which were uniformly subject to light traffic for the period of one year. The ratings were made visually according to the amount of raveling exhibited by the pavement and cannot lay claim to a precision greater than one unit. . . . Ratings 1 and 2 signify a very good performance, 4, 5, and 6 poor to very poor performance. The borderline cases are rated as 3 (5, p. 45).

In experiments conducted by Anderson, et al., the service rating showed only a fair correlation with results obtained in a standard loss of penetration on heating test. One asphalt which hardened as little as 12 percent had a service rating of 4, while another with a penetration drop of 20 percent performed excellently. The correlation of the service rating with the Resistance to Hardening value was, however, very high. All asphalts with a resistance to hardening value between 55 and 100 had service ratings of 3 or better, while asphalts with resistance to hardening values below 55 had poor service ratings.

In spite of the high correlation of the results of this test with in-service performance ratings of the asphalt, the test suffers from the disadvantage that the asphalt is heated to 160 C. In order to eliminate the effect of high temperatures, Anderson, Stross, and Ellings developed a test in which the oxidation takes place at 50 C.

> . . . the time necessary for an asphalt to drop to the critical penetration under standardized oxidizing conditions should be a measure of the durability of an asphalt. Since the attainment of the penetration would in

most cases take too long at the low test temperatures stipulated, it is necessary to develop a method of predicting this time from the behavior at shorter times. Direct extrapolation proved impossible, for it was found experimentally that the penetration is not a simple function of time.

The solution found was to combine the oxygen-absorption behavior of the asphalt, in amount and rate at a fixed time, with the hardening caused by a given oxygen absorption.

The oxidation is carried out in a stainless-steel bomb. . . .

The bomb is immersed . . . in an oil bath which can be regulated to 0.1 C. at the working temperature. For observation of the pressure, an automatic recorder, or for more accurate work, a Bourdon-type gage which can be estimated to 0.2 psi. can be used.

One hundred grams of the warm asphalt are poured into a 240-ml. (8-ounce) oil-sample bottle containing 67 grams of C.P. benzene. After weighing, the bottle is shaken until the solution is homogeneous; when it has reached room temperature, it is placed in the bomb, which is at 25 C. Next, the bomb is sealed and flushed with oxygen by charging it to 100 p.s.i.g. . . . and releasing it again. The flushing is repeated and the bomb is then charged with oxygen at slightly more than 100 p.s.i.g. After a short period (15 minutes) to test for possible leaks, the pressure is adjusted to 100 p.s.i.g. and the temperature is raised to 50 C. The pressure is recorded from now on, either continuously by automatic recorder or manually, as required for the plot described below. For each asphalt a run of 40 hours (or longer, for greater accuracy) . . . and several shorter runs of varying length are made. At the end of each run, the oxygen is released, and the asphalt is recovered as soon as possible to prevent its hardening in the solution. . . . The penetration of the asphalt is then measured. The data so obtained are used in the following manner:

The pressure drop is found by subtracting the observed gage pressure from the pressure of the original system at 50 C. This datum pressure calculated from the gas law and corrected for the solubility of the oxygen in the solution, the increase in vapor pressure of the benzene . . . is approximately 108 p.s.i.g. for the system under consideration. This value . . . was used in all calculations.

The pressure drop is plotted against time. . . . From this graph, the pressure drop at 40 hours and the slope of the tangent at this point·(in psi. per hour) are evaluated. The product of the pressure drop and the slope is called the oxidation rating. . . .

Next, the logarithm of the penetration after oxidation is plotted against the pressure drop. . . . The amount of oxygen needed to reduce the penetration to the value of 20 is obtained by extrapolation and is called the hardening rating and is expressed in psi.

The deterioration index indicative of the service behavior of an asphalt is obtained from the two quantities as follows:

$$\text{Deterioration index} = \frac{\text{oxidation rating}}{\text{hardening rating}} \times 100$$

The oxidation rating relates the oxygen consumption of the asphalt to time by multiplying the oxygen pressure drop at 40 hours by the rate of oxygen consumption at that time. . . .

The hardening rating was derived from the linear relationship which was found to exist between the oxygen consumption and the logarithm of penetration. . . .

The ratio of the two ratings gives the desired relation of critical oxygen consumption to time and . . . is expressed directly in psi. per hour; it therefore has the dimensions of a rate, and represents the rate at which asphalts deteriorate (5, p. 47-49).

In experiments carried out by the authors, this test correlated very well with the service ratings of the asphalt. A deterioration index lower than 15 indicates fully satis-

TABLE 2

PHYSICAL PROPERTIES AND SUSCEPTIBILITY TO OXIDATION OF
CHLORINATED AND UNCHLORINATED ASPHALTS

Asphalt	Percent increase in weight resulting from chlorination	Temp. R. & B., C.	Penetration, 0 C., 200 g., 60 sec.	Penetration, 25 C., 100 g., 10 sec.	Penetration, 25 C., 100 g., 10 sec.	Penetration, 46.1 C., 50 g., 5 sec.	Penetration, 25 C., 100 g., 5 sec. after 5 hours' heating	Penetration, 25 C., 100 g., 5 sec. after 10 hours' heating	Ductility, 25 C.	Ratio Pen. 46.1 C., 50 g., 5 sec. Pen. 25 C., 100 g., 5 sec.	Susceptibility Factor	Slope, log. time vs. log. penetration curve	Class (according to Pfeiffer)	Percent by weight soluble in carbon disulfide	Percent by weight insoluble in pentane	Loss of weight on heating 10 hours, percent	Loss of penetration on heating 10 hours, percent	Resistance to Hardening	Deterioration Index
A no Cl₂	44	37	82	107	352	75	69		100+	4.3	3.8	0.38	II	100	21.5	0.01	15.8	123	2.3
A 0.118	49	30	61	79	262	61	59		100+	4.3	3.8	0.37	II	100	23.3	0.01	3.1	269	1.9
A 0.495	58	9	23	29	92	24	24		24.5	4.0	3.6	0.32	II	100	27.1	0.01	-4.4		1.9
B no Cl₂	48	8	21	29	99	17	13		100+	4.7	4.3	0.46	I	100	26.2	0.04	38.1	15	19.8
B 0.383	55	2	15	19	66	15	15		100+	4.4	4.3	0.39	II	100	31.2	0.01	0		2.1
B 0.735	64	3	10	12	39	11	10		10.5	3.9	3.6	0.25	II	99.7	37.7	0.01	0		2.4
C no Cl₂	47	21	42	55	185	33	26		100+	4.4	3.9	0.41	II	100	23.8	-0.03	38.1	30	15.4
C 0.187	54	11	34	43	141	33	33		100+	4.3	3.8	0.38	II	100	26.1	0.01	2.9		5.3
C 0.431	62	7	15	19	61	14	16		34.5	4.1	3.7	0.33	II	100	30.3	0.01	-6.7		2.0
D no Cl₂	46	18	47	61	201	40	34		100+	4.3	3.9	0.39	II	100	22.4	0.01	27.6	48	13.1
D 0.236	51	22	45	58	189	43	40		100+	4.2	3.7	0.37	II	100	25.7	0.01	11.1	105	3.1
D 0.379	60	6	12	15	49	13	13		45.5	4.1	3.6	0.34	II	100	27.8	0.01	-8.3		2.0
E no Cl₂	44	19	63	84	277	44	31		100+	4.4	4.1	0.41	II	-	-	0.94	50.9	26	-
F no Cl₂	43	28	72	95	316	59	48		100+	4.4	4.0	0.40	II	-	-	0.91	33.3	48	-
G no Cl₂	46	26	65	84	273	54	45		100+	4.2	3.8	0.37	II	-	-	0.00	30.8	52	-
H no Cl₂	46	18	58	75	244	41	30		100+	4.2	3.9	0.36	II	-	-	0.01	48.2	28	-

factory performance. If the index is above 20, poor durability can be expected.

In the laboratory the resistance to hardening test was carried out in accordance with the procedure outlined by Anderson, Stross, and Ellings. In obtaining penetration data, the specimens were allowed to "cure" for five days as previously described. The results are given in Table 2 and Figure 1. Owing to lack of a stainless steel bomb, it was necessary to modify somewhat the procedure outlined for performing the oxygen bomb test. In place of the bomb described by Anderson et al., use was made of small compressed gas cylinders known as "lecture bottles." These cylinders which are ordinarily used to dispense compressed gases for laboratory demonstration have a capacity of about 250 ml. and are fitted with a removable valve to facilitate refilling. In performing the test, the cylinder valves were removed, and the asphalt solutions were poured into the cylinders. The valves were then replaced, and the cylinders flushed and filled with oxygen as described by Anderson. Bourdon-type gages with scales reading from 0 to 100 psi. were then attached to the valves. The cylinders were then immersed in a water bath at 50 C.

In all other respects, the test was performed in accordance with Anderson's procedure. The results are given in Table 2.

It should be emphasized that this test was performed with equipment differing from that used by Anderson, and that the
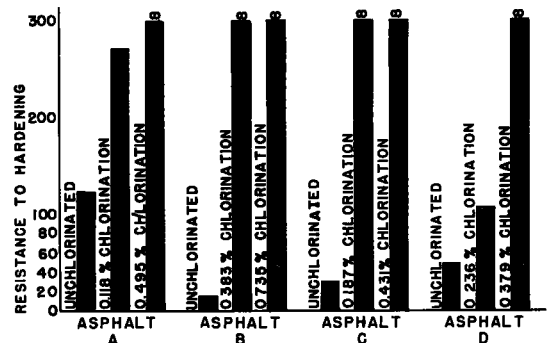
Figure 1. Resistance to hardening of chlorinated and unchlorinated asphalts.

values obtained from the deterioration index cannot be compared with those obtained by Anderson. Since it was impossible to standardize the equipment used with asphalts of known performance, the values obtained are purely relative.

Stripping Test

A simple stripping test known as the Tyler wash test was performed with asphalts A, B, C, and D, both unchlorinated and chlorinated, to determine the comparative susceptibility to stripping action. Limestone and chert were used as aggregates.

For this test, described completely by Tyler (14), a 500-gram sample of each aggregate was coated with 5 percent by weight of each asphalt. After a 24-hour curing period, the coated aggregate was separated into particles and placed in a jar with one liter of distilled water. The jar was then placed in a rack built to fit into a standard Ro-tap machine and shaken for 30 minutes. The aggregate was removed from the jar, allowed to dry, and rated for stripping on a ten-point scale. On this scale, 0 denotes no stripping, 1 to 3 slight stripping, 4 to 7 moderate stripping, and 8 to 10 excessive stripping. Table 3 gives average values based on the ratings of four observers.

TABLE 3

TYLER WASH TEST
Degree of Stripping from Chert and Limestone using
Unchlorinated and Chlorinated Asphalts

	Chert	Limestone
A – unchlorinated	3*	1
0.495% Cl$_2$	1	1
B – unchlorinated	2	1
0.735% Cl$_2$	1	1
C – unchlorinated	2	2
0.431% Cl$_2$	0	1
D – unchlorinated	2	1
0.379% Cl$_2$	1	1

*0 – 3, slight stripping; 4 – 6, moderate stripping;
  7 – 10, severe stripping.

COMMENTS

The changes in physical properties resulting from chlorination of the asphalts tested are in complete agreement with theory. With increasing amounts of chlorination, the penetration at a given temperature is decreased, the ring and ball softening point is increased, the percentage by weight of the asphalt insoluble in pentane is increased, the slope of the log. time versus log. penetration curve is decreased, the temperature susceptibility factor is decreased, and the ductility is decreased. These changes all indicate that when an asphalt is chlorinated there is a tendency toward the formation of a gel structure.

The results obtained in the loss of penetration on heating test (resistance to hardening) suggest that it may be possible to reduce materially the loss of penetration due to high temperature oxidation by chlorinating the asphalt. No asphalts containing an appreciable amount of volatile material were used in the chlorination experiments. Therefore it can be assumed that any loss of penetration on heating was the result primarily of oxidation.

Since all penetration tests were performed after a five-day "curing" period, and since the values thus obtained are considerably lower than when the test is performed according to AASHO standards, the resistance to hardening value of 55 used by Anderson to differentiate between asphalts which perform well and those which perform poorly does not apply. In the case of the results obtained in the laboratory, the critical value of resistance to hardening would be several points lower than 55.

The results obtained in the oxygen bomb test should not be considered conclusive.

The use of high temperatures during oxidation is avoided in this test, but the asphalt is dissolved in a solvent (benzene) from which it is later recovered. Although the benzene was distilled off under carbon dioxide, thus preventing further oxidation of the asphalt, it was impossible to be certain that all the benzene was eliminated. As explained previously, a very small amount of benzene remaining in the asphalt will cause a considerable increase in penetration.

According to results obtained in the Tyler wash test (Table 3), chlorination of an asphalt does not increase its tendency to strip from either a siliceous aggregate, such as chert, or a carbonate rock such as limestone. In fact, the data suggest that the opposite may be true; chlorination may increase the adhesiveness of the asphalt in the presence of water. If this is the case, it can be explained by the fact that chlorination increases the polarity of certain constituents of the asphalt.

CONCLUSIONS

1. Treatment of the asphalt samples tested in the laboratory with only a very small amount of chlorine (0.1 - 0.35 percent increase in weight of the asphalt) results in a very great increase in the resistance to hardening of the asphalt. Further treatment with chlorine does not result in a significant increase in resistance to hardening but may lower the ductility of the asphalt to a point where it is no longer useful for highway construction.

2. Chlorination of an asphalt does not increase its tendency to strip from mineral aggregates in the presence of water.

References

1. Herbert Abraham, "Asphalts and Allied Substances." 5th ed., New York: D. Van Nostrand, 1945, 2 vols.

2. Gene Abson, "The Softening of Oxidized Asphalts by Heating at High Temperatures and its Relationship to Oxidizing Temperatures and Recovery Temperatures," Proceedings of the Association of Asphalt Paving Technologists. Vol. 12, January 1942.

3. American Association of State Highway Officials, "Standard Specifications for Highway Materials and Methods of Sampling and Testing." Washington: The American Association of State Highway Officials, 1950. 2 vols.

4. American Society for Testing Materials, "1949 Book of ASTM Standards," vol. 3. Philadelphia, 1950.

5. A.P. Anderson, F.H. Stross, and E. Ellings, "Measurement of Oxidation of Stability of Road Asphalts," Industrial and Engineering Chemistry, anal. ed. vol. 14, 1942.

6. Arthur G. Bruce and John Clarkeson, "Highway Design and Construction." 3rd ed., Scranton, Pennsylvania: International Textbook Company, 1950.

7. Louis F. Fieser and Mary Fieser, "Organic Chemistry." 2nd ed., Boston: D.C. Heath and Company, 1950.

8. Samuel Glasstone, "Textbook of Physical Chemistry." 2nd ed., New York. D. Van Nostrand Company, Inc., 1950.

9. Laurence I. Hewes, "American Highway Practice." New York: John Wiley and Sons, Inc., 1942. 2 vols.

10. Highway Research Board, "Bibliography on Resistance of Bituminous Materials to Deterioration Caused by Physical and Chemical Changes," annotated. Washington: Highway Research Board, 1951.

11. H.G. Nevitt, "Temperature Control of Hot Mixes," Bituminous Roads and Streets, April 1952.

12. J.Ph. Pfeiffer, "The Properties of Asphaltic Bitumen." Amsterdam: Elsevier Publishing Company, Inc., 1950.

13. C.L. Shattuck, "Measurement of the Resistance of Oil Asphalts to Changes in penetration and Ductility of Plant Mixing Temperatures," Proceedings of the Association of Asphalt Paving Technologists. vol. 11, January 1940.

14. Owen R. Tyler, et al., "Adhesion of Bituminous Films to Aggregates," Engineering Bulletin, Purdue University, Research Series No. 62, Lafayette, Indiana: Purdue University Engineering Experiment Station, 1938.

15. B.A. Vallerga, "The Nature and Testing of Asphalts and Asphaltic Mixtures," University of California Syllabus Series No. 318, Berkeley: University of California Press, 1950.

Constitution and Characterization of Paving Asphalts

JAMES H. HAVENS, Research Chemist, and
W. F. DANIELS, Assistant Research Chemist, Kentucky Department of Highways

This paper contains results of analyses with asphalts of various composition
and derivation. The objective of the study is to define and correlate consti-
tution and fundamental character of asphalts in a manner significant to their
use in highway pavements.

Further confirmation of the existence of three discrete solubility fractions
in asphalts was obtained by extraction methods and by paper-partition chroma-
tography. Paraffin bodies were revealed by microscopic examination of thin
films. Asphaltene fractions showed the presence of particulate material by
electron microscopy and also revealed indications of peptized asphaltene bodies.
Oils dialyzed from asphalt exhibited fluorescence, when exposed to ultra
violet radiation, similar to components of the oil fractions obtained by extrac-
tion. Oven tests revealed increases in asphaltenes and resin fractions and
attendant hardening of the asphalt.

It is suggested that proper interpretation of asphalt composition is contin-
gent upon adequate means for measuring the fundamental shearing character-
istics and other rheological properties of the composite asphalt.

●IN 1908, Clifford Richardson, in a treatise which he chose to entitle "The Modern
Asphalt Pavement" (1), documented the current status of asphalt technology and quality
criteria. Many of the basic concepts which he promulgated are still worthy of some
reflection and review. He seems to have been one of only a few at that time who enter-
tained a profound interest in the composition and characterization of paving asphalts.
At that time native asphalts, such as Bermudez and Trinidad, comprised the primary
sources of paving material, while petroleum residues and distillates were used largely
as fluxes and solvents. It was known then that solubility in light naphthas varied with
different asphalts; that portions of the naphtha-insoluble materials were soluble in car-
bon tetrachloride; and that the remaining fractions, except for inert minerals and
elemental carbon, were usually soluble in carbon disulfide.

The naphtha-soluble fractions were first called "petrolene" and the insoluble frac-
tion called "asphaltene." Richardson elected to use these terms in the plural in order
to gain the inference of mixtures of compounds belonging to a solubility class. He also
suggested that the term "malthene" might preferably be used to designate the naphtha-
soluble portions because they resembled oily materials to which the term "maltha" had
been applied elsewhere, and that the term "petrolenes" be reserved for those portions
volatile at 325 F.

He proposed the term "carbenes" for those materials insoluble in cold carbon tetra-
chloride but soluble in carbon disulphide. He rejected the existence of a "resin" frac-
tion on the basis that alcohol-soluble portions of oils did not resemble resins in their
behavior toward reagents. Although he published analytical data intended to character-
ize asphalts on this basis, he did not apply the terms directly. He regarded the oil con-
stituents as being comprised of paraffinic, olefinic, and napthenic hydrocarbons and the
asphaltenes as being comprised of polycyclic aromatics. He differentiated petroleum
crudes by their yield of asphaltic residues or paraffin residues and wax contents.

In 1916, Marcusson (2), in Germany, criticized Richardson's scheme for fractiona-
tion, because it did not take into account the saponifiable portions and did not reflect the
chemical character of the separated constituents. He surmised that, besides the oily
constituents, asphalts contained products formed by oxidation, condensation, polymer-
ization and sulfurization.

He divided the transformed products into: (1) neutral petroleum resins, (2) asphal-
tene, and (3) asphaltous acids and their anhydrides. Acids and anhydrides were iso-
lated as water-soluble salts. Asphaltenes were precipitated from a benzene solution
of the asphalt by adding a large volume of naphtha. Resins were isolated by pouring

13

the naphtha solution through absorbant fuller's earth from which they were extracted by refluxing with chloroform. The naphtha effluent, on subsequent evaporation, yielded the oil fraction, and the chloroform solution yielded the resin fraction.

He characterized the recovered oils as resembling viscous, fluorescent mineral oils consisting of saturated and unsaturated hydrocarbons. On the basis of the formalite reactions, he concluded that the oil constituents were, at least in part, unsaturated and cyclic compounds. He found, in characterizing the resins, that they softened at the temperature of boiling water and that on heating in a naphtha solution they darkened, became insoluble, and assumed the character of asphaltenes.

Marcusson's work has served as a pattern for further development and refinement of analytical study of asphalts. Strieter (3) substituted pentane for naphtha in the precipitation of asphaltenes and ethyl ether for benzene and chloroform (or carbon disulfide) in extracting the resins from the fuller's earth. He assumed that the determination of asphaltous acid would usually be omitted from ordinary fractionation procedures and considered the essential fractions to be simply asphaltenes, resins, and oils.

Hoiberg, et. al. (4, 5), have extended and refined fractionation procedures to include differentiations of hard and soft resins and waxes; and observed further that asphalts deficient in resins are characteristically associated with poor serviceability, poor adhesion, and a "cheesy" appearance; while high asphaltene and high resin contents (favorable to dispersion of the asphaltenes) impart low-temperature ductility and good serviceability to the asphalt.

As early as 1924, Nellensteyn (6) attempted to explain the rheological behavior of asphalts on the basis of colloidal theory. Richardson had suggested that asphaltenes imparted body to the asphalt. The oil fraction, of course, is expected to exhibit pure Newtonian or purely viscous flow; and if the asphalt does exhibit non-Newtonian properties, these characteristics are attributed in some way to the presence of asphaltenes and resins. Mixtures of asphaltenes and oils differ greatly from mixtures of asphaltenes, resins, and oils. Without resins, the asphaltenes tend to be somewhat insoluble in the oil and to flocculate. The presence of resins seems to enhance their dispersion or mutual solubility, and the degree of interassociation is thought to the controlling influence in non-Newtonian properties. The resins are intermediate between oils and asphaltenes, both in structure and molecular weight, and may be considered either as a necessary link in a progression of dependent solubilities or as protective colloids.

The present study emanates from a desire to further characterize and correlate the fundamental properties of asphalts in a way that would be significant to their use in highway pavements. The study has been largely exploratory, guided by three general theses: (1) the composition of an asphalt is outwardly manifest in its physical properties, and the two can be correlated if expressed in fundamental terms; (2) the fundamental rheological properties of an asphalt provide the only rational criteria from which to judge its usefulness as an aggregate binder for paving; and (3) criteria for paving-mixture design must be developed on an equally fundamental basis before these properties can have significant meaning. Changes in asphalts with time and ambient temperatures are naturally of particular interest, because permanence is inferred as a quality sought in a pavement and because hardening of the binder is thought to contribute deterimental influences to the pavement, causing it to crack and unravel.

ANALYSES OF ASPHALTS BY SOLUBILITY FRACTIONS

Conservative estimates suggest that the number of specific compounds that might exist in an asphalt would be so overwhelming as to preclude any possibility of ever isolating or cataloging them in this manner. Fractionation into solubility classes or groups (such as oils, resins, and asphaltenes) conveys considerable significance as to the molecular structure of the compounds and their relative proportions within the asphalts. Any further differentiation of types of compounds within these general solubility classes would serve to further characterize the fraction as to its relative susceptibility to reactions such as those causing hardening. In this respect, the ultimate objective would be to ascertain the relative proportions of paraffinic, olefinic, or napthenic types of compounds comprising the oil fraction and, possibily, to characterize the resins more

specifically as to their compound structure and derivation.

The feasibility of fractionating asphalts, although guided by numerous published procedures, was approached with some skepticism. The procedure described by Strieter and patterned after Marcusson was selected for its simplicity but was appropriately modified to fit the convenience of the situation. N-heptane and n-hexane, instead of pentane, were used as solvents for the oils and resins.

A 5-gram sample was used. The hexane- or heptane-soluble asphaltenes were filtered out, washed and dried to constant weight, and the percentage was calculated directly. The filtrate containing oils and resins was washed through fuller's earth contained in a Soxhlet filter thimble. This filtrate, slightly amber in color, was evaporated to constant weight to obtain the oil fraction. The fuller's earth was then refluxed with ether in the Soxhlet apparatus for a period of several days to extract the resins. Resins were recovered from the ether solution by evaporating to constant weight.

The results of these analyses for seven asphalts used in the study are presented in Table 1. The table also shows the analysis of the same asphalts after "weathering" for 7 days in a forced-draft oven at 100 C. ($\frac{1}{8}$ inch film thickness).

TABLE 1

ANALYSIS FOR CONSTITUENTS OF ASPHALTS

(Unweathered and after 1 week of oven weathering at 100C., $\frac{1}{8}$-inch film)

Sample	Source of Crude and Method of Refining	77 F. Pen.	77 F. Duct.	Soft. Pt.	Asphal-tenes	Resins	Oils	Totals	Loss on Heating	Diel Const.
				F.	%	%	%	%	%	
					Unweathered					
S-0[a]	(Unknown)	75	150+	122	16.93	14 01	68.42	99.36		
A-1[a]	Mid Cont., St. Run	90	150+	113	15.02	14.59	66 42	96 03		2 89
F-1[a]	Unknown, St. Run	88	150+	115	13 93	13.82	68.99	96.74		
F-1	Unknown, St Run	88	150+	115	11.92	18.10	67.00	97.02		
D-1	Cal. St. Run	81	150+	115	12.30	23.90	57.80	94.00		3.39
B-1	Venez., St. Run	89	150+	118	17.80	22.90	53.70	94.40		2.99
C-2	Rocky Mt., Cracked	77	150+	119	24.40	18.50	52.10	95.00		2.69
E-2	Mid. Cont., Cracked	70	150+	112	19.40	14.30	70.80	104.50		2.99
					Weathered					
S-0		26	39 & 65	140	18.53	21.17	58.63	98.33	1.39	
A-1		21	26 & 30	139	19.26	22.02	59 69	100 97	1.25	
F-1		24	12 & 15	141	17.25	20.27	63.10	100.62	0.19	
D-1		12		153	21.70	25.50	49.60	96.80	1.08	
B-1		23		154	25.90	23.90	47.50	97.30	0.81	
C-2		11		198	31.20	15.50	52.90	99.60	1.05	
E-2		10		181	27.40	14.10	60.30	101.80	0.81	

[a] Asphaltenes determined as n-heptane insolubles, other samples, determined as n- hexane insolubles

The mere accomplishment of the fractionations was not particularly satisfying but was a necessary expedient to further study. The precision achieved was somewhat disappointing and may be adjudged from the summations of separated fractions. The accuracy of the separations, of course, can only be appraised from the standpoint of fraction purity. An attempt was made to evaluate fraction purity, as well as fraction character, by the use of paper-partition chromatography, the results of which are discussed elsewhere in the report.

The oven exposure was deliberately made severe in order to produce extreme hardening. It was suspected, or feared, that the accuracy of the fractionations would not reveal slight changes in composition, but the analyses after hardening showed increases in asphaltenes and reductions in oils in every case and little change in resins. Insofar as the asphaltenes and oils are concerned, the results conform with and confirm the concepts of composition that have appeared in the literature during the past several years. Susceptibility to hardening has been considered by Pauls and Welborn (7), on the basis of accelerated oven tests, to be variable with respect to source of crude and method of refining.

With respect to resins, it is possible that the rate of conversion of oils to resins was naturally balanced by the rate of conversion of resins to asphaltenes. This possibility of dynamic balance or equilibrium has been suggested by earlier theorists. For example,

it has been noted that the formation of asphaltic substances in lubricating oils is attended or even preceded by the formation of resin-like compounds and that their concentration may remain fairly constant while the asphaltene-like substances increase in concentration.
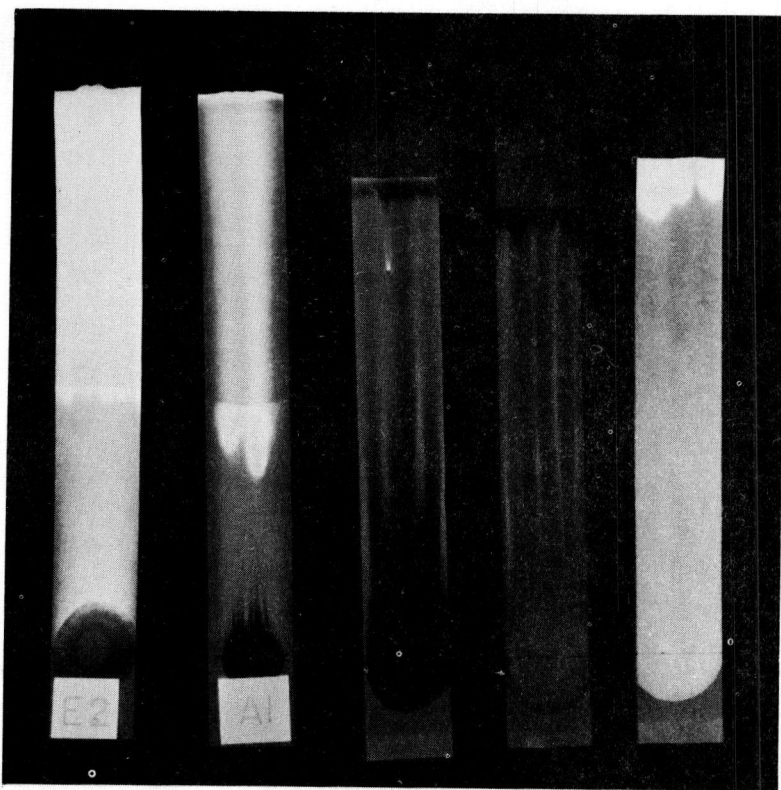

Figure 1. Typical chromatographs of composite asphalts and fractions. The two asphalts, E-2 and A-1 were developed about half way with hexane and butanol and then full length with acetone and methanol. The asphaltenes remained at the bottom, and resins developed half way up, and the oils developed to the top. The three unlabeled strips at the right are asphaltenes, resins, and oils separated from A-1, developed with benzene.

It is of interest to note, too, that the hardening process involved only a slight net loss in weight; this implies that either there was no appreciable evaporation of lighter constituents or else that the evaporation loss was compensated by oxygen absorption from the air.

STUDY OF CONSTITUENTS BY PAPER CHROMATOGRAPHY

As mentioned previously, the purity of the fractions and the discreteness of the boundary between the fractions was of some concern. It was felt that greater significance could be attributed to the fractionation if it could be demonstrated, at least qualitatively, that a recognizable boundary did exist or, if such did not exist, to characterize the boundary condition by some recognizable qualities. Paper-partition chromatography seemed to offer some interesting possibilities.

Basically, partitioning of complex mixtures by paper chromatography results from the combined influences of capillary migration of the solvent, the adsorptive attractions between the solutes and the paper, and the solubility of the solutes. Adsorption and degree of insolubility are assumed to exert restraint upon the movement of the solutes. By shifting the dynamic balance among these principal forces by a judicious selection of sol-

vents, the rate of migration of some components may be increased or retarded or may be caused to succumb to absorption and remain in situ.

Partitioning of complex mixtures by this method has been used rather extensively in studying certain biochemical processes, but its application to asphalt analysis originated within the past 2 years (8). During the course of the present study, Mariani (9), Herd (10), and Csanyi (11) have made independent chromatographic studies of this nature.

The value of partitioning is, of course, contingent upon a suitable method of identifying or characterizing the material after partitioning has been effected. In this case, fluorescence to ultra-violet light served as a natural distinguishing characteristic.

It was found that chromatographs of the oil fractions fluoresced a bluish white to a deep yellow and contained slight traces of orange; resin fractions, although predominately orange to brown, contained slight traces of yellow on the most advanced extremity and traces of black on the opposite extremity. Asphaltenes were predominately black but contained slight traces of orange and brown on the most advanced extremity. These observations suggest that discrete color boundaries do exist and, also, that the fractionation is not as discriminating as paper partitioning.

When benzene alone was used as a developing solvent for the composite asphalts, a continuous color spectrum from bluish white to black resulted. Differences among samples were easily recognized. The most-advanced oils of Sample No. B-1, a Venezuelan asphalt, fluoresced a bluish white, which is thought to be indicative of napthenic character. Pure paraffins are thought to be water clear and nonfluorescent. Complexity in molecular structure, therefore, is thought to be related to the progression in coloration from the blue side of the spectrum to black on the red side.

Using n-hexane as the developing solvent for the composite asphalts, it was found that the asphaltenes tended to migrate. Since they were assumed to be insoluble in this solvent in the fractionation procedure, there seemed to be a disparity in solvent selectivity in the two methods. Additions of n-butanol to the hexane imparted greater selectivity to the solvent and permitted the resins and oils to migrate without interferrence from the asphaltenes.

It was possible, too, to develop the oils and resins to some length with hexane and butanol and then to remove that solvent and to develop the oils further in a 1-to-1 mixture of acetone and methanol, leaving the asphaltenes and resins in situ. Without the methanol, acetone permitted resins and oils to migrate together. If acetone and methanol were used as the original developer, the oils migrated with the same selectivity.

In general, discrete partitioning of color boundaries could be achieved by coltrolling solvent selectivity. Just where these color boundaries should be established for fractional analyses is still somewhat a matter of conjecture. The fact that traces of orange appeared in the separated oil fractions, while an abundance of orange appeared in the resin fractions, leads to the conclusion that some interfractional contamination does result from the method used and that double precipitations and extractions may be necessary in order to obtain purity in the fractions.

It further suggests that the oil fraction should in all probability be considered to consist of three discrete subfractions: (1) the yellow-fluorescing portion; (2) the blue-fluorescing portion: (2) which are thought to be napthenic; and (3) nonfluorescing oils, which would be largely paraffinic in character. It also suggests that the resins consist of at least two subfractions, since they seem to consist in part of an orange-fluorescing material contrasted against a material somewhat brownish in color.

Typical chromatographs of developed composite asphalts and fractions are illustrated in Figure 1. The strips here were $1\frac{1}{2}$ inches wide and 12 inches long. Although the photograph was made with ultra-violet light, the separation of components is inadequately portrayed, unless viewed in true color. The sample to be developed consisted of 0.2 cc. of solution (0.2 gm. of asphalt in 50 cc. of benzene) placed dropwise about an inch from one extremity of the strip. Development was carried out by suspending the strips, sample end down, in a closed jar containing just enough of the desired solvent to touch the lower ends of the papers. Full development was usually accomplished in about 4 hours. The chromatographs were then dried of solvent and viewed under light of 3600 A. It was not possible, however, to preserve them for any length of time without

incurring significant losses in fluorescent brilliance of the lighter constituents.

The selectivity of solvents, although differing somewhat when used as a chromatographic developer as compared with use as an extractant, was guided in a general way by accepted rules of solubility as applied to organic analysis (12): (1) A substance is most soluble in a solvent to which it is most closely related structurally.   (2) As one goes higher in any homologous series derivatives exhibit properties more and more like the parent hydrocarbon. (3) Compounds of very high molecular weight exhibit decreased solubility in the inert solvents even though the solvent and solute may be of the same homologous series.

Predictions of solubility must, however, be considered from the standpoint of dielectric properties of solute and solvent. A solvent of high dielectric strength, for example, would tend to be a poor solvent for a low-dielectric solute. On the other hand, a solvent differing radically in structure but having similar dielectric strength may prove to be an effective solvent. Benezene, for instance, is usually an effective solvent for asphalt and paraffinic hydrocarbons, although differing greatly in structure. Solubility predictions have also been extended to include a consideration of similarity in surface tension.

Insofar as asphalt solubilities are concerned, it may be summarized that the greater the departure in structure from the paraffinic type of compound the more deficient in hydrogen the compounds become, and in the higher molecular weights, increasing hydrogen deficiency is indicative of more-highly condensed cyclic or aromatic structures attended by reduced solubility (13, 14).

DIALYSIS STUDIES

Sample A-1 was dialyzed with benzene in a combined osmoticdialysis cell, shown in Figure 2, for a period of 6 months. After about 2 weeks, the benzene began to exhibit some bluish-white fluorescence, which increased steadily throughout the remainder of the period.   Under white light the benzene first assumed a slight yellow coloration, which progressed steadily to a deep amber. At first, the bluish-white fluorescence was taken as an indication that only napthenic and paraffinic types of oils were being permitted to diffuse through the membrane; however, subsequent chromatographic studies of the benzene solution revealed the presence of the yellow-fluorescing component typical of the oil fraction.   Since the possibility of transformations occuring within the dialyzed oils during this length of time can not be completely discounted, the yellow component cannot be attributed exclusively to diffusion.   Actually there was a slight indication of asphaltenes registered on the chromatograph, which would be even less likely to occur as a result of diffusion. Considering these factors, it is not quite possible from this experiment to assign a specific selectivity or permeability limit to the membrane.

If there were any osmotic pressures occuring during the period, they were so small in magnitude that they could not be observed or measured in the cell used. Osmotic pressures, however, are sometimes considered to be a measure of the forces that attend diffusion. Since the observed rate of diffusion was extremely slow, it may not be so surprising that the osmotic pressure could also be extremely low.

This interest in osmotic pressures is based on their compliance with the fundamental gas law, $PV = nRT$, in which n is the mole concentration of solute or, in the case of colloids, the equivalent concentration of particles having the same kinetic energy as a molecule. Solutions normally exhibit higher osmotic pressures than colloids because of inherent differences in particle weight.   As a hypothetical case, consider a liter of asphalt containing 200 gm. of asphaltenes and exhibiting osmotic pressure in the order of 1mm. of Hg. , then the mean theoretical particle weight would be in the order of 75 million. In contrast, consider that only 20 gm. of sucrose in a liter of water is sufficient to exhibit over 100 cm. of Hg. pressure while the mean theoretical particle weight, on the same basis as in the case above, is in order of 350, which is in close agreement with its accepted molecular weight.

MICROSCOPIC STUDY OF THIN PREPARATIONS

Although no attempt was made to isolate solid paraffins in the fractionation proced-

ure, microscopic examination of thin prep-
arations by dark-field and oblique illumina-
tion revealed a structural quality which
might be attributed to the presence of solid
paraffins. All seven of the asphalts studied
exhibited these bodies to some extent.

Figures 3 and 4 are bright-field photo-
micrographs of thin preparations, approx-
imately 1000X magnigication, showing high-
ly refractile bodies which appear much
lighter than the matrix material. Under di-
rect examination through the microscope,
they appear prismatic or crystalline.

In the early days of petroleum asphalts,
according to Richardson, some crudes
yielded asphaltic resuidues so high in wax-
es or solid paraffins that they tended to
scale, due to crystallization, and to concen-
trate on the surface. These effects were
considered to be a detriment to both adhe-
sion to aggregate and to internal cohesion.
Now, by the use of dewaxing processes,
similar crudes are probably used indiscrim-
inately; but the amount of wax tolerated in
the asphalt is usually in the order of 3 to 5
percent or less. The presence of fairly
large percentages of waxes is usually ap-
parent from specific volume-temperature
curves, because they may exhibit a volume
change of as much as 10 percent over their
melting-point range.

Figure 2. Combined dialysis-osmotic-pres-
sure cell.

According to Pfeiffer (15) paraffin-wax content is still considered by some particu-
larly in Europe , to be closely related to quality of the asphalt; however, this view is
probably taken in regard to adhesion and the effect that the paraffins may have on tem-
perature susceptibilities.

ELECTRON MICROSCOPY

The rheological behavior of asphalts, particularly with respect to their degree of de-
parture from strict Newtonian flow, suggests that at least some of them are not true
solutions and that their behavior can only be explained on the basis of internal structure.
In general, it is now assumed that the oils constitute the continuous phase and the as-
phaltenes the disperse phase, while the resins serve as peptizing agents (the degree of
peptization of the asphaltene bodies being the principal factor imparting elasticity and
thixotropy to non-Newtonian asphalts.

Katx and Beu (16) failed to find indications of the existance of a distinguishable as-
phaltene micelle by electron microscopy but concluded that asphaltenes may be "poten-
tial colloids." In other words, they could be viualized as undergoing molecular aggre-
gation or dispersion, depending upon their degree of solubility or insolubility in the oils
with which they are associated.

Figure 5 shows a typical view of Sample S-O, at a magnification of 6,900X, in which
the oily phase is quite apparent and separate from the black, presumably solid, asphalt-
ene and resin bodies. In the more-concentrated areas, the large flakes may possibly
be platy wax, and there is a somewhat vague, but very real, impression of fibrous struc-
ture. Figure 6, shows an area selected from the center of Figure 5 magnified to
41,400X. Unfortunately, however, resolution was not improved due to the thickness of
the sample; but the fibrous quality of the structure has been made more apparent. The
darker fringes surrounding the black bodies may be indicative of a more-highly concen-
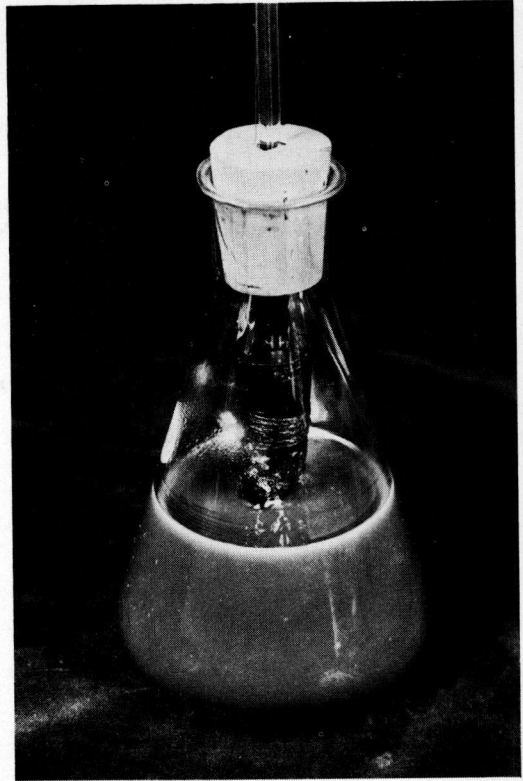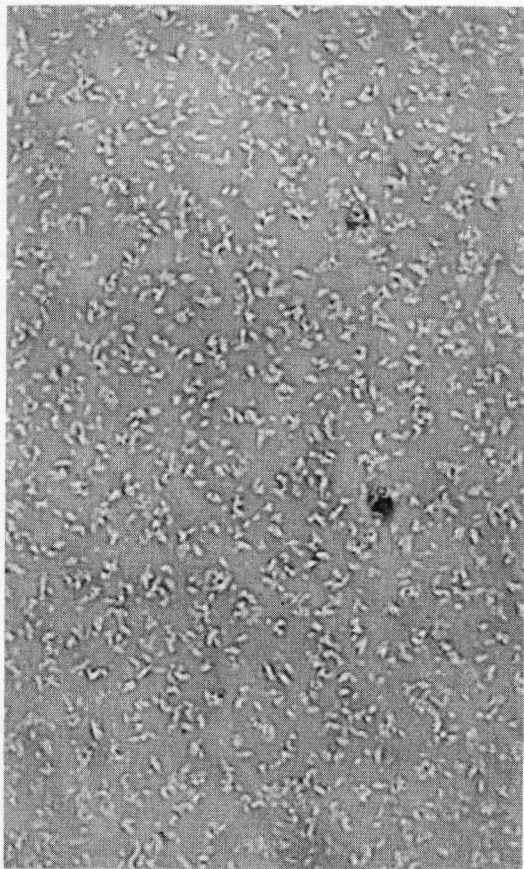
Figure 3. - Photomicrograph of sample C-2, brightfield, magnification approx. 1000X.

should be expressed in equally fundamental terms. For the sake of clarity, it may be of interest to consider the following generalized version of some classic, though perhaps elementary, concepts applied to the flow properties of matter. They are best illustrated graphically as in Figure 8.

If a material possesses elastic properties as portrayed above, its elastic deformation is proportional to the stress applied (stress/strain = a constant, called modulus of elasticity). If Δstress/Δstrain departs from a constant with increasing stress, the deformation is not elastic but permanent and is called shear (re-arrangement of molecular units) or plastic flow.

trated fibrous aggregation, the dark bodies themselves being the floculated or precipitated asphaltenes and resins. Since the films here were prepared from a benzene solution, they may not represent an equilibrium condition of dispersion.

In Figure 7 a similar preparation was shadow-cast with vaporized chromium and is shown at a magnification of 17,500X. The black bodies convey the impression of a completely flocculated system, and the oily phase seems to have migrated out into the chromium film.

In a generally way, at least, these studies seem to confirm the existence of structure in asphalts.

RHEOLOGICAL STUDIES

If composition of an asphalt is to be correlated with its rheological properties. the tests must describe a fundamental physical property of the material and

Figure 4. - Photomicrograph of Sample S-O, brightfield, magnification approx. 1000X.

to zero rate of shear, should yield zero stress.

If a similar plot of stress versus rate of shear should yield a linear relationship which failed to intercept the origin when extrapolated to zero rate, then the material is considered to be plastic and to have an elastic threshold to shear and complies with the equation $n' = \frac{(F - f)}{R}$. The point of intersection on the stress axis would then indicate the magnitude of the elastic component of the stress. Here, where Δ stress/Δ rate of shear = n', n' has the same dimensions as the vicosity coefficient (n) above. Both express fundamental physical constants of materials and are variable only with respect to temperature. The threshold stress (f) is also a fundamental material constant describing a discrete physical property.

Experimental conformity to either of these idealized concepts would serve to characterize an asphalt as purely viscous or purely plastic. Disconformity would mean that the material possesses visco-elastic properties of somewhat greater
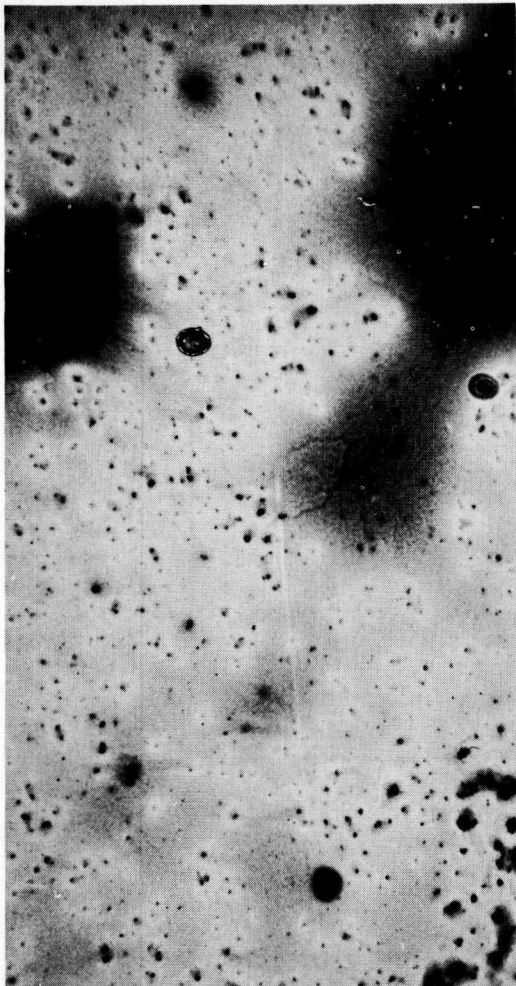

Figure 5. Electron micrograph, asphalt film Sample S-0, magnification 6,900X.

Eventually a point is reached where Δ stress/Δ strain is zero, and the ratio ceases to describe one as a function of the other. Then the material is presumed to be within a region of pure plastic flow and undergoing equilibruim shearing. The summation of deformations first involves an elastic component and then a shear component. This is then an idealized plastic, sometimes referred to as the Bingham solid (17).

A viscous material, or pure Newtonian liquid, is by definition one in which rate of shear is proportional to stress; and Δ stress/Δ rate of shear = F/R = a constant (n) called the coefficient of viscosity. Thus, if stress and rate of shear are the quantities measured, they should yield a linear relationship when plotted to arithemetic scale; and on extrapolation

Figure 6. Electron micrograph, Sample S-0, magnification 41,400X.
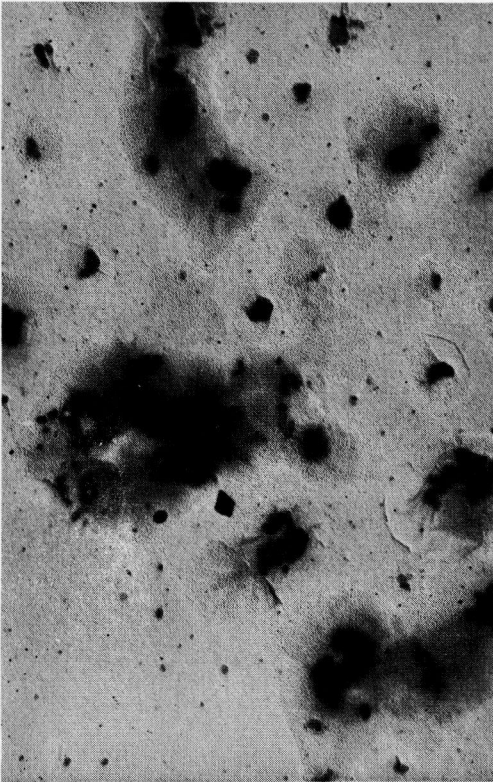
Figure 7. Electron micrograph, Sample S-0, magnification 17,500X, shadow-cast.

complexity, which may or maynot be definable (18). Other anomolous flow characteristics have been recognized. In some cases, it has been found necessary to include an abstract exponent in the Bingham equation in order to correct for nonlinearity. An apparently viscous material, for instance, may have internal elastic elements visualized as discontinuous springs acting in a viscous medium or as Maxwell's mechanical model, consisting of a spring and dash-pot connected in tandem (19).

Plastic materials may be considered to have a permanent but deformable internal structure, while thixotropic materials have internal structures variable states of development and decay with respect to time and environmental conditions. Thixotropy would not be revealed as a discrete property of a material from the relationship as discussed above and would have to be evaluated on the basis of time versus rate-of-deformation relationships under constant stress in order to describe such decay and development of structure. In the case of the Bingham solid and the pure Newtonian liquid above, deformation rate (R) necessarily represents equilibrium rates of shear or constant rates of shear with respect to time.

Although this treatment may be an oversimplification of concepts, it should be pointed out that the real difficulties involved in an experimental study or evaluation of the rheological behavior of asphalts arise from deficiencies in measuring insturments. In
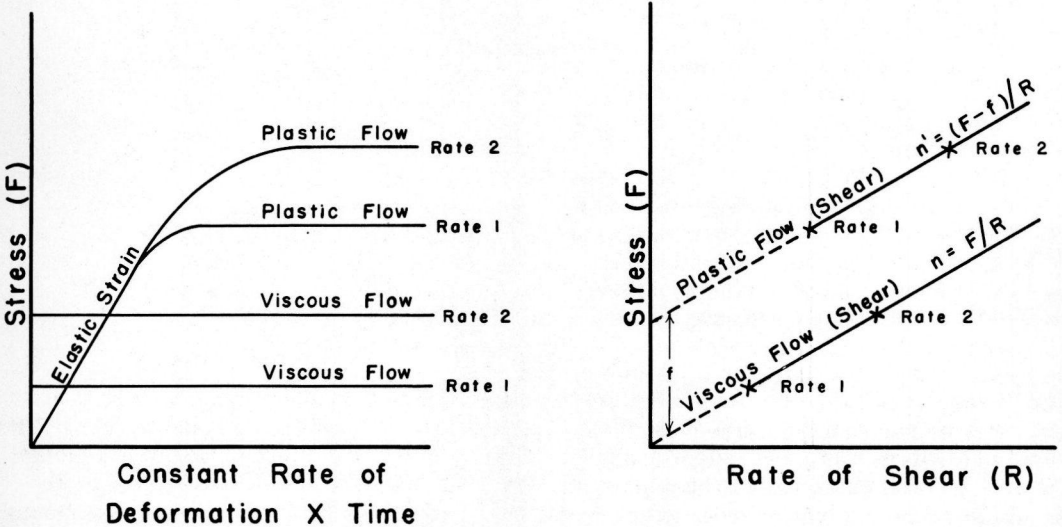

Figure 8. Graphical representation of fundamental-flow characteristics of matter.

a prototype instrument, it would be necessary to preserve a constant shearing area throughout the duration of the test and to
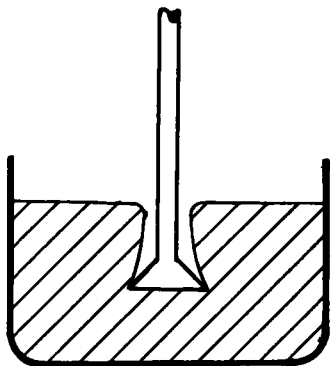

Figure 9. Schematic Drawing of Loading Device.

Figure 10. Plot of time versus depth of penetration, for all samples, 62.5 gms. on 1 sq. cm.

be able to measure and record rate of shear under various selected shearing loads and temperatures. None of these conditions are fulfilled by the standard penetrometer test, although Mack (20) and a number of others (21) were surprisingly successful in applying correction factors in an effort to interpret it on this basis. The rotary viscometer as used by Traxler et. al. (22) and other modifications of the rotary concentric-cylinder types (23) seem to offer the advantage of constant shearing area.

Figure 11. Relationships derived between stress and rate of shear, 77 F., original asphalt. Percent of asphaltenes and standard penetration is shown for each asphalt.

In this study, however, the penetrometer was used as a loading and measuring device; but instead of using a standard needle, a truncated cone-shaped bearing-plate type of loading system was used (see Figure 9). All surfaces were amalgamated to prevent adhesion. The loading area was approximately 1 sq. cm. It was not intended to revise the penetration test by this means at all but, rather, to find some expedient means of measuring deformation or shear at somewhat slower rates and to do it in a way that would yield a reasonably linear relationship with respect to depth of penetration and time.

For extremely hard asphalts, a bearing area of approximately 0.08 sq. cm. was used. This was necessary in the case of Samples E-2 and C-2, after hardening in the oven, in order to be able to conduct the test in one day and in order to overcome the threshold stress with a reasonable amount of load on the penetrometer stem.

Thus far these tests have not been made at temperatures other than 77 F. The linearity between depth of penetration and time may be adjudged from the data for one stress level plotted in Figure 10. It was observed that deeper samples and larger sample and larger sample cans yielded linearity over greater depths of penetration. In other words, it was suspected that boundary geometry of the sample may account for some of the departure for linearity.

A mean rate of shear over the approximate range of 5 to 10 mm. of penetration depth was estimated. Mean rate of shear was then plotted against load, as discussed earlier. This was done for the seven asphalts reported in Table 1. The experimental relationships derived (See Figures 11 and 12) are thought to be fundamentally characteristic of the asphalts. It should be borne in mind that these data represent extremely slow rates of shear and involve only about a threefold increase in rate. The resulting linearity is, therefore, not necessarily contradictory to other work reporting departures
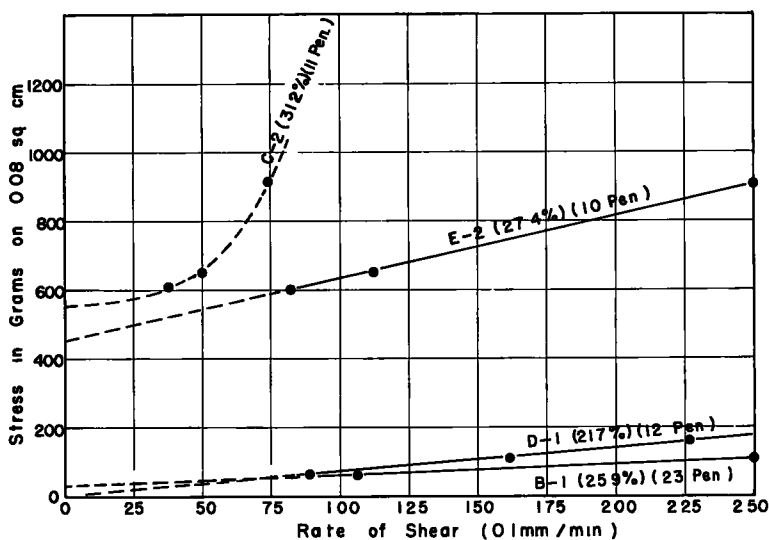
Figure 12. Relationships derived between stress and rate of shear, 77 F., after oven hardening, four asphalts only.

from linearity over ten-to thirty-fold increases in shear rates.

It is quite apparent from Figures 11 and 12 that fundamental rheological behavior of these asphalts is related to, or at least dependent upon, their constitution. It is quite apparent, too, that standard 77 F. penetration fails to differentiate the samples in any intelligible manner. Inasmuch as concentration of the asphaltenes represents the major difference in fractional constitution of the samples studied, it would be expected to reveal attendant rheological manifestations. However, it may not be concluded that these manifestations are not colligative with respect to the resin and oil fractions. The degree of aromaticity of the resins, as considered by Hillman and Barnett (24), may favor a higher degree of dispersion of the asphaltenes, whereas a highly napthenic or paraffinic disperse medium may tend to flocculate the asphaltenes and reduce their effective concentration.

Samples C-2 and E-2 are purportedly cracked asphalts, the origin of S-O is unknown, and the other four are purportedly straight run materials. Only limited significance can be attached to the extrapolated intercepts in Figure 11, because there was some deviation within the data from which the lines were drawn. Apparently, at least, there is a threshold stress (f) associated with Samples C-2, E-2, B-1, and D-1. The

slopes of the lines, even if considered only as apparent viscostities, tend to increase with increasing asphaltene content.

In Figure 12, the two cracked asphalts are clearly differentitated from the two straight-run materials, the threshold stress is quite unmistakeable, and the two asphalts have become more plastic as a result of hardening in the oven. It is not known whether the inflected curve drawn for Sample C-2 is real or spurious. One other observation, however, has a significant bearing on these interpretations:

After these samples had been " dimpled" in the test, it was noted that Samples D-1 and B-1 flowed as a result of the differential "head" surrounding the depression; and there was no trace of the dimple after 24 to 48 hours. The two cracked asphalts retained the dimple in its original shape for several weeks, and the samples had to be melted down in order to restore their original smooth surface. The fact that the two straight-run asphalts smoothed-out means that they flowed under infinitesimal stresses, which clearly characterizes them as viscous liquids.

SUMMARY AND CONSLUSIONS

A cursory knowledge of asphalt constitution enhances basic understanding of their rheological behavior and susceptibility to age hardening. Collectively, these factors are capable of describing the fundamental character of near-solid asphalts and are essential considerations in the development of quality criteria and in establishing specification requirements. This study reemphasizes the need for testing methods that directly reflect fundamental properties of the asphalt and which may eventually afford a rational interpretation of the significance of these properties in paving mixtures. If the mechanical properties sought in a bituminous pavement were sufficiently delineated on an equally fundamental basis, then the criteria for judging the significance of binder properties would be much clearer.

ACKNOWLEDGEMENT

REFERENCES

1. Richardson, Clifford, The Modern Asphalt Pavement, Wiley and Sons, New York, 1908.

2. Marcusson, J. "The Chemical Structure of Natural Asphalts," Chem. Abs., Vol. 11, p. 1291 (1917).

3. Strieter, O. G., "Method for Determining the Components of Asphalts and Crude Oils," RP 1387, Jour. Res., N.B.S., Vol. 26, p. 415 (1941).

4. Grant, F. R., and Hoiberg, A. J., "A Practical Method of Analyzing for the Constituents of Asphalts," Proceedings, Assoc. Asph. Pav. Tech., Vol. 12, p. 87 (1940).

5. Hoiberg, A. J., and Garris, W. E., "Analytical Fractionation of Asphalts," Ind. and Engr. Chem., Anal. Ed., Vol. 16, p. 294 (1944).

6. Nellensteyn, F. I.; "Constitution of Asphalt, "Jour. Inst. of Petroleum Technology, Vol. 10, 1924.

7. Pauls, J. T., and Welborn, J. Y., "Studies of the Hardening Properties of Asphaltic Materials, " Public Roads, Vol. 27, p. 187 (1953).

8 Gregg, L. E., and Alcoke, W. H., "Investigations of Rubber Additives in Asphalt paving Mixtures," Proceedings, A.A.P.T., Vol. 23, p. 28 (1954).

9. Mariani, Eugenio, "Application of Paper Partition Chromatography to Petroleum Analysis," Jour. Inst. of Pet., Vol. 39, p. 240, (1953).

10. Herd, Magnus, "A Paper-Strip Method of Examining Fuel Oils S uspected of Being Indentical," The Analyst, Vol. 78, p. 383 (1952).

11. Csanyi, L. H. and Fung, H., "A Rapid Means of Determining the Constituents of Asphalts, "Proceedings, A.A.P.T., Vol. 23, p. 64 (1954).

12. Kamm, Oliver, Qualitative Organic Analysis, 2d. Ed. , Wiley and Sons, New York, 1947.

13. Cruse, Wm. A. , and Stevens, D. R. , The chemical Technology of Petroleum, McGraw-Hill, New York, 1942.

14. Ellis, Carleton, The Chemistry of Petroleum Derivatives, Vol. II, Reinhold, New York, 1937.

15. Pfeiffer, J. Ph. , The Properties of Asphaltic Bitumen, Elsevier, New York, 1950.

16. Katz, D. L. , and Beu, K. E. , "The Nature of Asphaltic Substances," Ind. and Eng. Chem. , Vol. 37, p. 195 (1945).

17. Bingham, E. C. , Fluidity and Plasticity, McGraw-Hill, New York, 1922.

18. Report on the Principles involved in the Determination of Absolute Viscosity, prepared by A. S. T. M. Committee E-1 on Methods of Testing, Proceedings, A. S. T. M. , Vol. 51, p. 441 (1951).

19. Alfrey, Turner, Jr. , Mechanical Behavior of High Polymers, Interscience Publishers, Inc. , New York (1948).

20. Mack, C. , "The Flow Properties of Asphalts and Their Measurements by the Penetrometer," Trans. J. Soc. Chem. Ind. , Vol. 58 p. 306 (1939).

21. Mill, C. C. and Harrison, V. , "A Note on the Measurement of Flow Properties by the Penetrometer," Trans. J. Soc. Chem. Ind. , Vol. 59, p. 66, (1940).

22. Traxler, R. N. , Schweyer, H. E. , and Romberg, J. W. , "Rheological Properties of Asphalts," Ind. and Eng. Chem. , Vol. 36 (1944).

23. Symposium on Methods of Measuring Viscosity at High Rates of Shear, Special Technical Publication No. 111, A. S. T. M. , Philadelphia, Pa. , (1951).

24. Hillman, E. S. , and Barnett, B. , "Constitution of Cracked and Un-Cracked Asphalts," Proceedings, A. S. T. M. , Vol. 37, p. 558 (1937).

Action of Microorganisms on Petroleum-Asphalt Fractions

S.J. BURGESS, Laboratory Technician
Montana State Department, Helena, Montana

Sometimes while doing the commonplace, one of our senses is momentarily conscious or aware of an intangible thought or reflex. If vivid enough, it might dimly remind us or have a slight connection with something in the past. Failing to recognize or grasp its importance is usually of no great significance. But on rare occasions we stumble upon something of merit. The inception of this study originated in such a manner, and has brought together two sciences which heretofore had been comparative strangers in highway laboratories.

Associating the characteristic odor of butyric acid with the rancid odor of old oil mats aroused the first suspicions of a bacterial action. Previous research into the causes of butterfat rancidity furnished sufficient background of bacteriology to recognize this fermentation in old asphalt mats. In searching for corroboration of this theory in available asphalt literature, none could be found. However, a search in other fields proved very fruitful.

The first attempts to invite bacteria to live on asphalt were crude and, in the light of later developments, were somewhat humorous. At the outset, the idea was contradictory, because it is a generally accepted opinion that bacteria were instrumental in the formation of our underground petroleum pools. It was also difficult to concieve how bacteria could help make it if they destroyed it and how anything would want to eat a substance that is generally accepted as being unpalatable. However, investigation points out few organic substances which are not acceptable sources of food for bacteria or their enzymes.

The first important thing this study brings out is a new conception of asphalt oxidation that has taken place as the result of what now might be called improper design. Secondly, to emphasize asphalt as one of the most durable and versatile materials available, when properly used.

There are sufficient tables and supporting data included for students of bacteriology who might be interested in serious study of the subject. A comprehensive set of tables is included for petroleum engineers interested in the analysis of the asphalts.

The rate of breakdown in the various categories of oils ranging from kerosene to heavy residues is interesting. The prerequisities of microbial action on the asphalt fraction attacked is the major topic.

However, no estimation of how much damage is attributed to microbial action is attempted in the paper. Various types of procedure for laboratory tests are of necessity given in detail to enable those interested to do their own testing. Contrary to the usual rule, there is little value in pictures in this particular instance, but a few were thought necessary to show some of the peculiar results.

Surprising results are found in the extracted asphalt of old oil samples, leading one to believe that there is a great deal that could be done to improve petroleum products by direct treatment with specific types of bacteria.

The paper also points out the reworking of old mats that have had bacterial action in progress on the shoulders in poor practice, because the entire mat becomes contaminated. Chemical inhibitors are also discussed. The paper has little to offer in support of the "syneresis theory" as being a contributing factor in the hardening of asphalt.

● THIS investigation of the action of soil bacteria on asphalt was undertaken for the purpose of proving whether bacteria oxidized asphalt to the extent that bacterial action

TABLE 1
PHYSICAL PROPERTIES OF OIL FRACTIONS STUDIED

Oil No.	Description	Centistokes Viscosity		Estimated Composition[a]			Calc.[b] Mol. Wt.	Est. Ave. No. Rings Per Molecule
		At 210°F.	At 100°F.	%A	%N	%P		
1	Filtered 185 Pa. Neutral	5.93	39.86	9	15	76	407	1.7
2	Portion of (1) Distilled	3.56	17.76	9	16	75	328	1.4
3	Portion of (1) Distilled	5.91	40.37	8	16	76	402	1.7
4	Portion of (1) Distilled	9.66	86.03	9	14	77	504	2.0
5	Portion of (1) Extracted	8.39	120.9	29	13	58	347	2.4
6	Portion of (1) Extracted	4.86	28.19	0	24	76	377	1.5
7	Portion of (1) Extracted	7.00	44.69	0	17	83	459	1.3
8	Penna. Bright Stock	32.91	540	9	13	78	740	2.9
9	Final Raffinate from (8) Extracted	103.8	2321	0	(14)	(86)	1194	(3.1)
10	Portion of Bosco Neutral Extracted	11.1	350.8	54	19	27	270	3.2

[a] Values obtained using method of Vlugter, et al. (1935). % A = % aromatics; % N =% naphthenes; % P=% paraffins.
[b] Obtained using a modified Keith and Roess method (1937).

could be considered one of the main factors of asphalt oxidation. This supposition was against traditional thinking, because it did not conform to the accepted views of petroleum-research chemists on asphalt oxidation.

In less than 2 years, the partial oxidation of asphalt and the oxidation of the oily fraction of asphalt has been definitely accomplished in the laboratory by exposure to soil microorganisms. However, on account of this being a comparatively new field of investigation, much more time and work is needed before this type of oxidation can be properly evaluated. Therefore, this paper is naturally limited and should be considered as a preliminary report.

The asphalt industry has devoted a great deal of work and much thought to the cause of oxidation of certain types of asphalt in our highways, but the question is debatable as to whether or not all the causes have been identified, insofar as being able to duplicate in laboratories the same oxidation which takes the life out of the asphaltic binder on the highway.

The very fact that asphalt mats, in most cases, must be placed on the ground in the enviroment of soil bacteria should have been of more concern in the past, especially where moisture conditions in the interface of the mat and base is ideal for the activity of bacteria.

Less than 100 years ago, chemists, biologists, and plant physiologists were not agreed on the role of soil bacteria, especially the chemists who held experiments by biologists in contempt, since chemists regarded their science as capable of covering all phases of chemical change. Until very recently, the science of bacteriology has been a stranger in a chemistry laboratory. However, it has come to the attention of some petroleum geologists as a possible means of discerning gaseous hydrocarbons in deep wells. Recognition of petroleum microbiology is now becoming highly important to petroleum refining in light of recent investigations in the use of bacterial enzymes as catalysts.

A search of literature has revealed only a fragmentary study of the action of micro-

organisms on petroleum asphalt. Microbiologists evidently shunned these heavier hydrocarbons, because they could not be satisfactorily dispersed in an aqueous solution.

One explanation of why we have not recognized this new concept of oxidation before or why it has not been of general exchange of knowledge is because there has been little or no fratenizing between microbiologists and petroleum engineers. Otherwise, it is difficult to account for the laco of such pertinent information. Bacteriologists were not too concerned with the meaning of damage done to petroleum by bacterial action but, rather, with the questionable effect of the several petroleum products on the growth of bacteria. Had microbiologists realized the important significance of this, they no doubt would have conveyed it to petroleum engineers. It has been the general thought among some petroleum engineers that most any petroleum product would kill bacterial growth, and it would be more than likely that others have not given bacteria a thought.

Excavations of ancient cities of the world have revealed the use of asphalt from natural deposits as bonding mortar for stone and brick work, a water-proofing for stone cisterns, joint filler in stone pavement, etc. The remarkable part that asphalt played in the ecomony of that era is the more remarkable, because it has been found in such a perfect stage of preservation. California has asphalt deposits that yielded the remains of prehistoric animals that existed 200,000 years ago. This should be proof enough of asphalts durability. So why should we question it now? Perhaps we have not always used asphalt to the best advantage, nor have we understood certain limitations of its use, because the asphalt of the Romans that is found in a good state of preservation was used in heavy films of $1/4$ inch or more. It is hoped this study will help to answer some questions on asphalt oxidation.

OCCURRENCE

Fortunately not all oil mats are subject to attack by soil bacteria, mostly because conditions are unfavorable to bacterial activity, such as a lack of moisture at the interface of mat and base, or because of man-made barriers, such as curbing used in street paving preventing the bacterial migration at the edge of the mat. Better design of base course has eliminated moisture entrapment under that mat to a large extent.

There has been no precedent to follow in this investigation, and some manner of laboratory testing had to be devised to determine whether bacteria or its enzymes con-

TABLE 2

FERMENTATION OF OILS AT ROOM TEMPERATURE

Bacterial plate counts in millions per ml. of one percent oil dispersed in a mineral salt medium

Type of Oil	Time of Permentation in Days							
	0	1	2	4	6	8	10	14
Penna. Crude	13	65	300	380	220	121		
Mich. Crude	8.3	44	420	680	780	290		
Oil No. 1	0.6	35	640	510	380	390		25
Oil No. 2	1.3	65	620	970	720	740		90
Oil No. 3	0.6	88	350	400	540	670		104
Oil No. 4	1.0	47	180	165	210	370		99
Oil No. 5	0.6	121	440	310	300	280		71
Oil No. 6	0.1	270	390	690	140	610		270
Oil No. 7	0.3	14	510	1470	1150	880		39
Oil No. 8	1.2		90	240	91		240	180
Oil No. 9	0.4		6	12	21		51	36
Oil No. 10	1.1		55	87	250		108	157
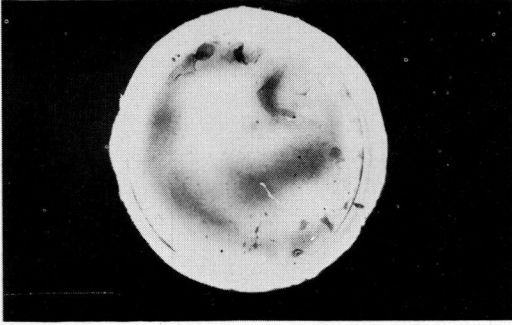
Control No. 1 Extracted Oil From Mexican Straight Run on Sterile Salt Media. Color is greenish orange.
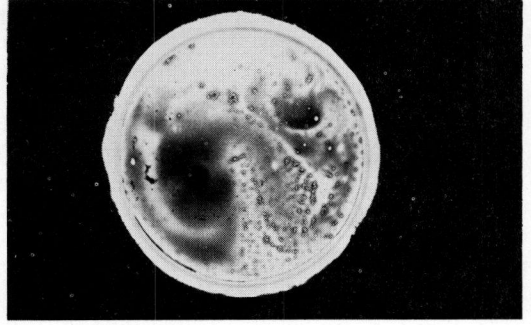
Control No. 2 Extracted Oil From Cracked Asphalt on Sterile Salt Media. Color is greenish orange.

Dark Portion - Remains of Oil Film. The Results of Oil No. 1 on Inoculated Salt Media —Color is gray to Biege Light gray is fatty acid residue.

Dark ragged remains of Oil Film. The Results of Oil No. 2 on Inoculated Salt Media—Color is brown to black. Light gray residue is fatty acid.
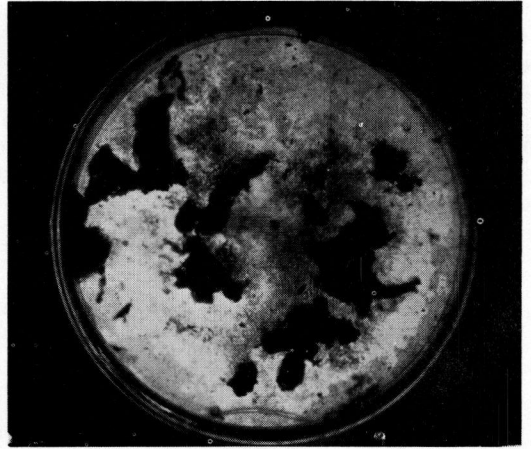
Figure 1

tributed either directly or indirectly in the oxidation of petroleum asphalt used for binder in asphalt-aggregate mixes for highway surfacing.

A noticeable odor or rancidity aroused the first suspicions of a bacterial action in oil mat specimens. To date, this has not been duplicated in experiment with oil mat out in the field, but the same rancid odor has been obtained in laboratory tests. Just what particular fatty acid gives off this rancid odor has not been determined and will have to await further investigation.

No tests to date have proven bacterial action on oil mats laid primarily for the specific purpose of proving bacterial attack, because there has not been time to set up experimental work. Nevertheless, laboratory tests have shown a definite bacterial action on asphalt, so it is only a logical deduction that comparative results can be duplicated in the field under favorable conditions. In the following study an attempt will be made to show that laboratory results could have a relationship with field work and are, therefore, an indication of the same oxidation of asphalt that will be found in field tests.

TEST PROCEDURE

It is relatively simple to set up tests for experimenting with bacteria and asphalt to prove the oxidation or decomposition of the oils by bacteria. A source of suitable bacteria can be found in most any garden soil. Ordinary garden soil contains from 50,000

to 200,000 bacteria per gram. Old oil-soaked soil will materially hasten the whole process, because of the relatively large amount of hydrocarbon oxidizers present to clean up the oil.

Dispersion of the asphalt in the media is the toughest problem. Glass cloth is a good vehicle, because it can be weighed before and afterward. Thin films can be made by diluting the asphalt with benzene and then allowing evaporation before covering.

Ottowa sand could be a feasible means of getting a large surface area for oxidation, the whole idea being to expose enough asphalt in order to have a sufficient quantity left for analysis. Using 4 g. of SC-3 on 100 g. was not a sufficient quantity for later analysis. The briquettes lost some stability and appeared dry.

Considerable work has been done on the direct oxidation of asphalt by soil bacteria in this laboratory, but it is little compared to the amount needed before any definite conclusions can be drawn. With all the irrefutable evidence available on the subject, using light hydrocarbons, there is still a lack of substantitating data on the utilization of the very-heavy hydrocarbons by microorganisms.

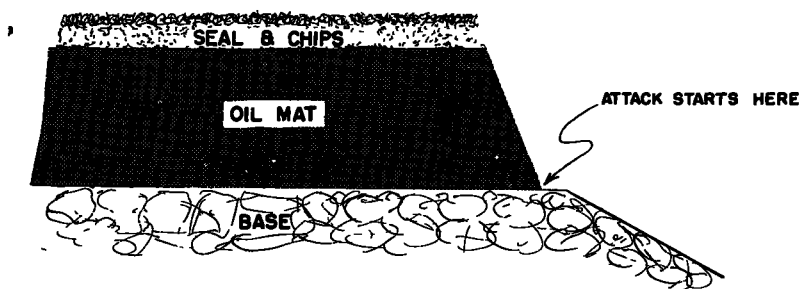## CYCLE OF PENETRATION

NEW CONSTRUCTION

Figure 2

Up to date, the several tests with SC-3 and the extracted oils of asphalt have been conducted, but there has not been time to duplicate these tests, which is essential for verification of data.

Likewise, the field samples which have been studied on the work on these field samples is in the same category. Because field conditions are difficult to control, under the best of circumstances, data from this source are unreliable.

The ordinary oiled road with sweet clover or grasses growing on the shoulder next to the oil mat has all the requisites for microbes to attack its edges. Plant growth indicates cellulose of dead grass roots as nitrogen to furnish the initial energy. The bacteria live on this while adapting the oil fraction of the asphalt as food. The utilization of this oil is slow to begin with, but with favorable temperature and oxygen at the interface of the mat with the base, it flourishes until made dormant by the lack of proper temperature or other unfavorable conditions.

Everything else being equal, the rate of asphalt oxidation by microorganisms appears to be in direct proportion to its viscosity or penetration and is evidently showed as the concentration of the oily medium is reduced, either by refinery distillation or the process of oxidation by microorganisms, to a point where insufficient oil is present for food and energy.

The most-simple and readily understood explanation of the action of microorganisms on the oil components of asphalt is the ability of microbes to utilize the oil as a source of energy and food, their most-outstanding characteristic being their ability to create a favorable enviroment out of one that is hostile to their growth. For instance, a fairly heavy concentration of phenol, which is considered a germicide, can be utilized as a food after alterations.

TABLE 3

OXYGEN UPTAKE AND CARBON DIOXIDE EVOLVED IN OIL DISSIMILATION

Oil	Trial	Time of Incubation Days	Oxygen Uptake	CO$_2$ Evolved	CO$_2$ (Corrected)	Ratio CO$_2$/O$_2$
			ml.	ml.	ml.	
	1	6	11.80	7.81	7.42	0.63
1	2	5	11.06	7.70	7.31	0.66
	3	5	9.77	6.62	6.29	0.64
	1	5	6.82	4.52	4.29	0.63
5	2	5	7.32	5.14	4.88	0.67
	3	5	7.21	4.77	4.53	0.63
	1	5	10.89	7.81	7.42	0.68
7	2	5	11.23	8.14	7.74	0.69
	3	5	9.77	6.94	6.59	0.67
	1	8	6.17	2.92	2.77	0.45
8	2	8	7.50	3.63	3.45	0.46
	3	8	4.23	2.09	1.99	0.47
9	1	13	2.17	0.42	0.40	0.18
	2	13	3.08	0.24	0.23	0.07
Control (1)	1	5	0.24	0.16	0.15	
	2	5	0.17	0.11	0.11	
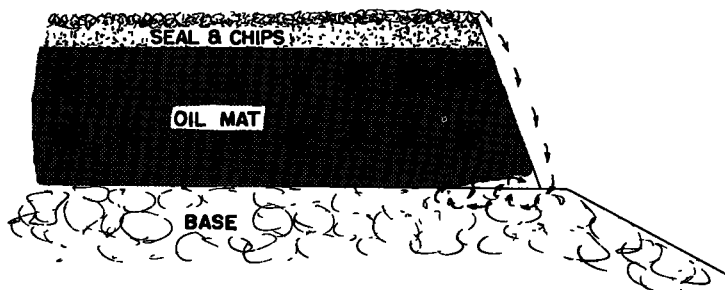
Figure 3.

So far, asphaltenes have not been found to be an acceptable source of hydrocarbon for microbial utilization, and it is doubtful that resins, which are also a polycyclic aromatic compound, can be utilized. Therefore, the oil seems to be the most-attractive portion of asphalt. Once this is consumed to the point where the asphaltenes and resins shield the remaining oil, bacterial growth is halted for lack of a suitable source of hydrocarbon.

The procedure of verifying the action of microorganisms on hydrocarbons is relatively simple and requires a minimum of equipment, care, and time.

The first step is the procurement of a well-inoculated aggregate from under an oil mat or some of the oil mat itself. To the inexperienced operator, this might sound slightly difficult, since the presence of hydrocarbon oxidizers is not visible. So a good rule to follow to find such an aggregate would be the selection of an oil mat, preferably

an SC-3 or MC-3 several years old, which has broken edges. Since moisture is a pre-requisite for the growth of microorganisms, it would follow that an oil mat through farm or meadow land would have more than an average bacteria population in the underlying base.

For ease of sampling, pieces of oil mat already cracked or broken off of the shoulder are ideal. It will also be noted that there is a particularly rancid odor to these pieces of broken oil mat when slightly heated.

The next step is to mix up a nutrient balanced salt solution. Put in 200 g. of the above aggregate and allow to stand 48 hours at room temperature or better still at 103 deg.

CRACKED & BREAKING AWAY
ALLOWS MOISTURE & OXYGEN
UNDER NEW PORTION
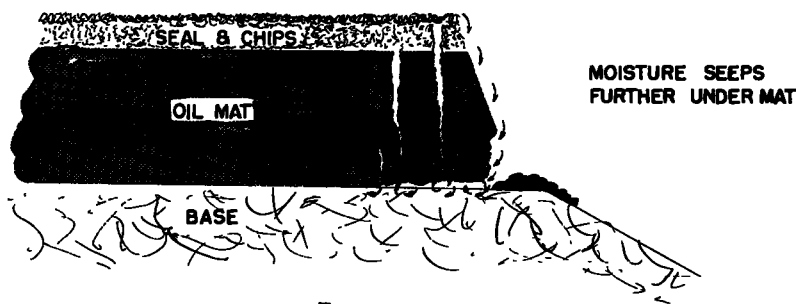
Figure 4

After standing, agitate slightly to get the bacterial slime in suspension; then pour this solution into a large flat container. The container or dish should be covered to prevent evaporation after a thin (this is important) film of asphalt has been poured on the surface of the salt solution. Care must be taken in the selection of the asphalt. A straight-run asphalt has a lighter gravity than the cracked products and will float, being

TABLE 4

ORGANISMS ISOLATED FROM OIL FERMENTATION

Group	Morphology on Agar	No. Isolated	Glucose	Litmus Milk	Nitrate Red'n	Gelatin Liquefaction	Oil Media	Probable Genus
I	Green, flat, spreading	63	A	RP	+	Stratiform	F	Pseudomonas (aeruginosa)
IIa	White, con-vex, mucoid	46	A	A or —	±	—	A	Achromo-bacter
IIb	White, con-vex, mucoid	26	A	R	±	Slow or —	F	Achromo-bacter
IIIa	White, mu-coid, some-times brown-ish	28	—	RP	±	—	A	Alcaligenes (radiobacter)
IIIb	White, mu-coid, some-times brown-ish	52	—	Al	—	—	F	Alcaligenes
Misc.	Reds, yel-lows, whites, etc.	35						Mixed

TABLE 5
EFFECT OF COMBINED OXYGEN ON BACTERIAL GROWTH
IN THE PRESENCE OF AIR

Hydrocarbon	Medium	Coryne-bacterium Simplex	Proacti-nomyces Species (17A)	Pseudo-Monas Strain No. 6	Pseudo-Monas Strain No. 8	Pseudo-Monas Pyocy-aneus No. 58	"Cul-ture X"
Light oil	Complete	+++	+++	+++	+++	+++	0
Kerosene	Complete	0	0	+++	++++	++++	+++
Light oil	Minus NO$_3$	+++	+++	+++	+++	+++	0
Kerosene	Minus NO$_3$	0	0	+++	++++	++++	+++
Light oil	Low PO$_4$[a]	+++	+++	++	++	++	0
Kerosene	Low PO$_4$[a]	0	0	+	+	++	+
Light oil	Minus SO$_4$	++++	+++	+++	+++	+++	0
Kerosene	Minus SO$_4$	0	0	+++	+++	++++	+++
Light oil	Minus all	+++	+++	0	+++	++	0
Kerosene	Minus all	0	0	+++	++++	+++	+++

++++, excellent growth; +++, good growth; ++, moderate growth; +, slight growth.
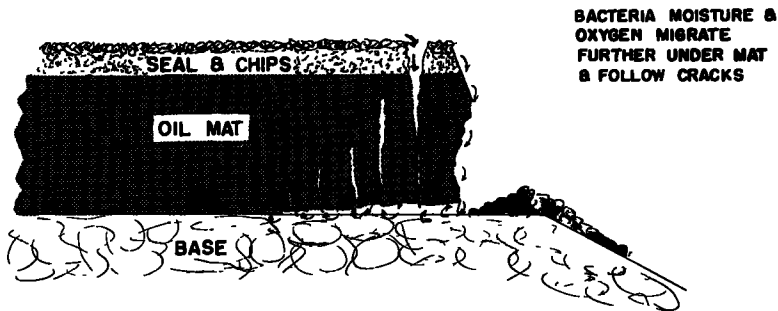
MIGRATION OF BACTERIA, MOISTURE & OXYGEN

Figure 5.

lighter than water. After a few weeks, the film of asphalt will submerge. If it is desirable to prevent complete submergence of the asphalt film, a piece of plastic screen supported by pieces of glass will retain the film and will aid in removing the asphalt for analysis at the conclusion of the test. The control does not need this precaution.

Several other methods can be suggested, such as impregnating glass cloth with asphalt and burying the cloth in an inoculated soil. The main objection to this is the small amount of asphalt available for final analysis. At room temperature or slightly higher, 50 percent of the asphalt will have disappeared in about 8 weeks. As the asphalt hardens, the action becomes slower until it finally levels off at its equilibrium, providing the same temperature is maintained.

Another visible test is that of identifying the gas given off from a closed container of inoculated oil mix. The gas production of this is measured by the displacement of water in a condenser jacket by the gas given off by the decomposition of the hydrocarbons by the microorganisms. Analysis by an orsat gas analyzer will identify the product as CO_2, CO, etc.

These accelerated tests are suggested as procedures to shorten the time element involved; because if field conditions were simulated in the laboratory, it would take months to obtain the same results which are achieved by acceleration in weeks, as mat temperatures are comparatively low.

TABLE 6

CHANGE IN pH PRODUCED BY CULTURES ON VARIOUS HYDROCARBONS

	In Respirometers				In Flasks	
	Ps. pyo-cyaneus No. 58	"Culture X"	Pseudo-monas strain No. 8	Cory. simplex	Pseudo-monas strain No. 8	Cory. simplex
Culture medium alone	6.98	7.10	6.90	6.89		
Control—no hydrocarbon	6.60	N[a]	6.87	6.85	6.87	6.90
Gasoline	6.66	6.82	N	N	N	N
"Skelly-solve"	N[a]	6.85	N	N	N	N
Raw Kerosene	5.65	6.61	6.57	6.81	5.45	6.19
Treated Kerosene	5.81	6.78	6.57	6.65	5.45	5.79
Light oil	5.50	6.88	6.53	6.59	5.92	5.85
Heavy oil	6.52	N	6.70	6.59	6.53	6.26
Paraffin wax	6.15	N	6.69	6.71	6.63	6.33

[a]N = no growth.

RAVELING CAUSES LOSS OF SUPPORT
UNDER REMAINING MAT

RUBBLE PUSHED FROM
UNDER MAT CAUSING
LOSS OF SUPPORT &
MAT BREAKS OFF
CYCLE THEN STARTS
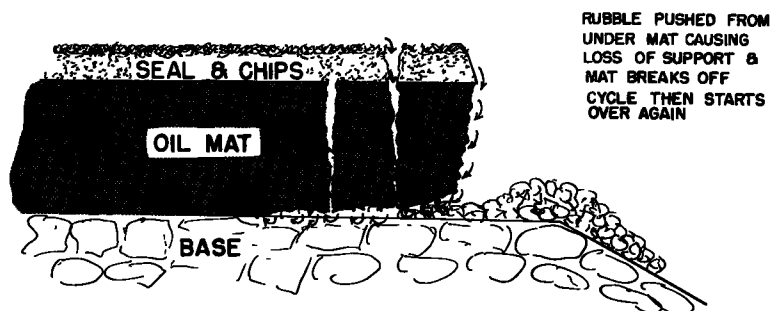OVER AGAIN

SEAL & CHIPS

OIL MAT

BASE

Figure 6.

Those that might undertake these experiments will be surprised how quickly the action of the soil microorganisms effect the appearance of the asphalt film as the oily constituents are dissipated. And it is important to run the controls under sterile conditions, in order to have an accurate comparison. While this study was originated by the writer, there is always reason to believe that others are working with the same idea in mind but, probably, with a different approach and differing procedures.

It should be pointed out that some precaution is necessary in handling these accelerated tests, since the nutrient media and above average temperature can and do produce harmful bacteria, especially if a nutrient agar is employed for making cultures for microscopic examination.

Also, some care is necessary to keep the controls free of contamination; the dishes should be washed thoroughly and kept covered to prevent any soil particles or drops of cultured solution from them.

The first experiment set up consisted of several 2-inch briquettes made of -10 material and oiled with 270-pen, asphalt, immersed in a covered jar which contained soil and water enriched with salts. The soil was taken from the capitol lawn under the grass roots. The gas from the jar was discharged into limewater; the precipitate indicated CO_2. After 6 months, the briquettes were taken out and the condition noted. Three of

TABLE 7

DISTILLATION TESTS ON KEROSENE AFTER BACTÉRIAL ACTION
(P. pyocyaneus culture No. 58)

Percent Distilled	Control (Aerated)	Control (Not Aerated)	No. 58 (Aerated)	No. 58 (Not Aerated)
	Initial b. p.			
	120°	116°	120°	120°
	b. p.	b. p.	b. p.	b. p.
10	193	190	189	184
20	199	196	195	194
30	202	201	201	202
40	211	205	206	204
50	217	215	213	215
60	223	222	214	221
70	227	228	226	229
80	211	213	232	223
90	225	223	243	243
End point	232	229	257	248

the briquettes had disintegrated, but four were in fair condition, except for a blistering effect on the exterior. The specimens were dried and the asphalt extracted. On examination the asphalt was found to be quite hard (76 pen.). The amount recovered was not sufficient to obtain more data, such as ductility.

Another one of the first set-ups was a platter with a thin film of SC-3 floated on water which had been inoculated with the culture. A control was set up for comparison on sterile salts.

Out of the 35 g., only 13 g. were recovered from the cultured platter. The asphalt had hardened to the extent of having a softening point of 85 F. The control flowed at room temperature, at which 31 g. were recovered. A very-small amount adhered to the platters in both cases.

Care was taken in recovering the asphalt, so there would not be any change due to heat; drying was done at 150 F.; a water bath was used to prevent overheating while distilling of the CCL₄. I should mention that it had been dissolved in CCL₄ and filtered through gooch to remove any soil particles. The control was treated in a similar manner (Table 8).

TABLE 8

ANALYSIS OF THE RECOVERED OILS
FROM SC-3

	Control	Bacteria Treated
Insolubles	.05	.08
Asphaltenes	16.97	21.71
Oils	43.95	31.51
Ether Resins	14.98	19.84
Acetone Resins	22.73	25.11
Total	98.68	98.65

CARBON-HYDROGEN RATIO

	Control	Bacteria Treated
Hydrogen	10.25	9.96
Carbon	85.16	84.40
Sulfur	Not run	Not run
	Flows at room temp.	85 deg. softening point

A second set up was run on MC-3 straight run. This time a larger amount of road oil was used in order to have a larger amount left. At the end of 30 days, the control was taken off, likewise the asphalt that had been exposed.

No attempt was made to make a complete analysis of the results, but the asphalt exposed to bacteria had hardened to the consistency of an MC-6 having a penetration of 283 at 77 F. The control was unchanged.

Perhaps the most-instructive project has been the collecting of the CO_2 gas as it is given off at 1,000 g. of oil mix inoculated with culture. A desiccator with clamps was used to contain the oil mix. An outlet at the top is connected to a condenser jacket full of water. As the gases collect, they force out the slightly acidiz-

ed water. This method can show rate of gas production if temperature is fluctuated.

In a recovered asphalt from an old SC-3 mat, the gravity of the asphalt was 1.16 and was 9.64 percent soluble. This is an extremely high gravity, although the ductility and the penetration at 77 F. was 100 and 60, respectively.

OXIDATION OF OILS

The oxidation of the extracted oily component of both straight-run and cracked asphalt by bacteria or its enzymes is relatively fast. Approximately one gram of oil spread as a film on the inoculated salt media shows signs of dissipation in 3 to 6 days. Straight-run oils are oxidized more completely than cracked, as shown by Figure 1.

NEW CONSTRUCTION WITH MEMBRANE

MEMBRANE SHEDS TOP WATER
& WILL NOT ALLOW SEEPAGE
BACTERIA CULTURE CANNOT
GROW FOR LACK OF MOISTURE
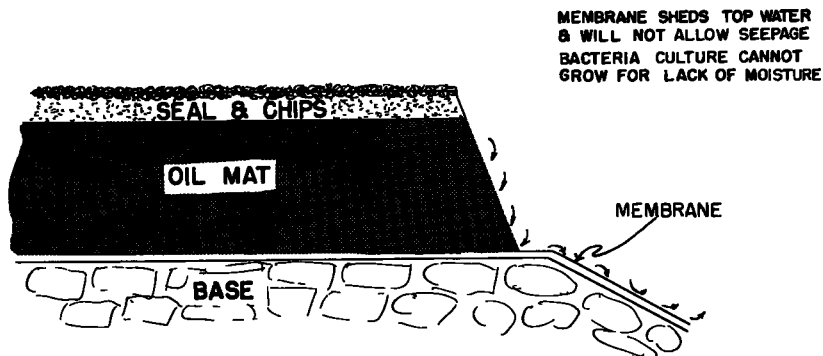
SEAL & CHIPS

OIL MAT

MEMBRANE

BASE

Figure 7

A waxy substance with a melting point of about 140 F. appears on the surface of the media from the straight-run oil in approximately 30 days. After several months this changes to a white substance. The film of slime that submerges gradually disintegrates until it has a ragged appearance. The oils from cracked asphalt retain a much darker color and a heavy body. Speculating on the cause of this will have to suffice until a sufficient quantity of oil is on hand for experiments. Because of the different origin of these oils, their behavior is most important to future study.

MINERAL SALTS MEDIUM

The most-successful method of isolating organisms capable of utilizing hydrocarbons is the use of a mineral-salt hydrocarbon enrichment medium in which the hydrocarbon is the only source used, with minor modifications, by Sohngen (1913), Tausz and Peters (1919), Tauson (1929), Buttner (1926), Haag (1926), Jensen (1934), and Gray and Thornton (1928). The basic-salt medium given in Table 9 proved to be quite satisfactory. The medium is adjusted to ph 7.0 to 7.2 with dilute NaOH. Two percent of washed agar is added whenever a solid medium is needed.

FIELD STUDIES

One of the biggest obstacles in field sampling is reliable data on the road oil used. It is necessary to know the complete analysis of the cutback asphalt. In a great many cases when maintenance crews have worked over an oil mat, there is only a small fraction of the information available. It is not sufficient to find a big difference in the penetration of the extracted

TABLE 9

Water, distilled	1000.0
$MgSO_4$	0.2 g.
$CaCL_2$	0.02g.
KH_2PO_4	1.0 g.
K_2HPO_4	1.0 g.
NH_4NO_3 or	
$(NH_4)_2 SO_4$	1.0 g.
$FeCL_3$	2 drops conc. sol.

asphalt from samples taken in different sections of the oil mat, such as the big differ-
ence between shoulder and centerline samples.

There are so many factors to be taken into consideration that anything definite, in
the way of exact duplication, is practically impossible. Therefore, it is safer to ap-
proach the study with the idea in mind of duplicating, as nearly as possible, field con-
ditions in the laboratory, where they can be controlled. This eliminates any influence
of aggregate absorptions or chemical reaction from the aggregate, unknown pH of mois-
ture, and such variables as extremes of temperature.

CRITERIA OF OXIDATION AND OXIDATION RATE

Criteria of hydrocarbon utilization are easily recognized by the production of CO_2;
change of pH by acid formation and saponification; change of specific gravity; and loss
of quantity. In Table 3 is the oxygen-uptake chart, which shows the oxygen consumed
as always being less than the volume of CO_2 produced. Light hydrocarbons use more
than the heavy ones. Turbidity is an indication of bacterial action. It is the belief of
some investigators that dead cell substance is one of the contributing factors which in-
crease the specific gravity of asphalt that has been worked on by bacteria, acting like
a heavy mineral colloid.

In Table 1 is a description of the hydrocarbons used by Stone, Fenske, and White
in their work, ranging from light hydrocarbons to fairly heavy residues. The rate of
oxidation varies according to the several governing factors, but several workers have
given the figure of 0.7 ml. per sq.decimeter in 24 hours on mineral oil.

The paving asphalts seen have a great advantage over the road oils, because the
film thickness on the aggregate can be heavier. And since there is no cutback to be
lost either by aggregate absorption or evaporation, the initial film is quite permanent. The
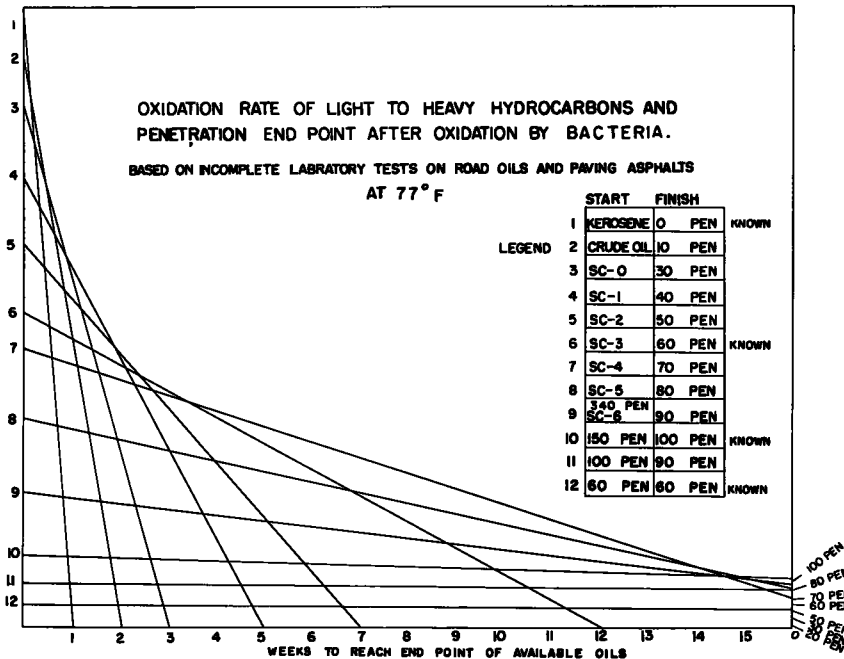
Figure 8

second advantage of paving asphalts is their comparative freedom of light ends that pro-
vide initial nutrient to microorganisms.

While this might be the first time that the investigation of bacterial action on road
oils and cutback asphalts has been attempted, it is hoped that others will become inter-
ested in the potentials of this new concept of oxidation.

Since the rate of bacterial attack depends on the accessibility of oil present, paving

asphalts were not used in this study to any extent due to this hardness and low oil con-
tent, rendering them practically immune to microbial attack and no dispersion possi-
bilities in an aqueous media.

There is a tendency to underestimate the importance of film thickness. This is the
all-important factor, because all results are dependent on this. While it may not be
possible to duplicate film thickness in successive tests, it is essential to obtain a uni-

OXIDATION CURVE OF LIGHT TO HEAVY HYDROCARBONS TO
PENETRATION END POINT AFTER METABOLISM BY BACTERIA

LEGEND		START	FINISH	
	1	KEROSENE	0 PEN	KNOWN
	2	CRUDE OIL	10 PEN	
	3	SC-0	36 PEN	
	4	SC-1	40 PEN	
	5	SC-2	50 PEN	KNOWN
	6	SC-3	60 PEN	KNOWN
	7	SC-4	70 PEN	
	8	SC-5	80 PEN	
	9	340 PEN SC-6	90 PEN	
	10	150 PEN	100 PEN	KNOWN
	11	100 PEN	90 PEN	
	12	60 PEN	60 PEN	KNOWN

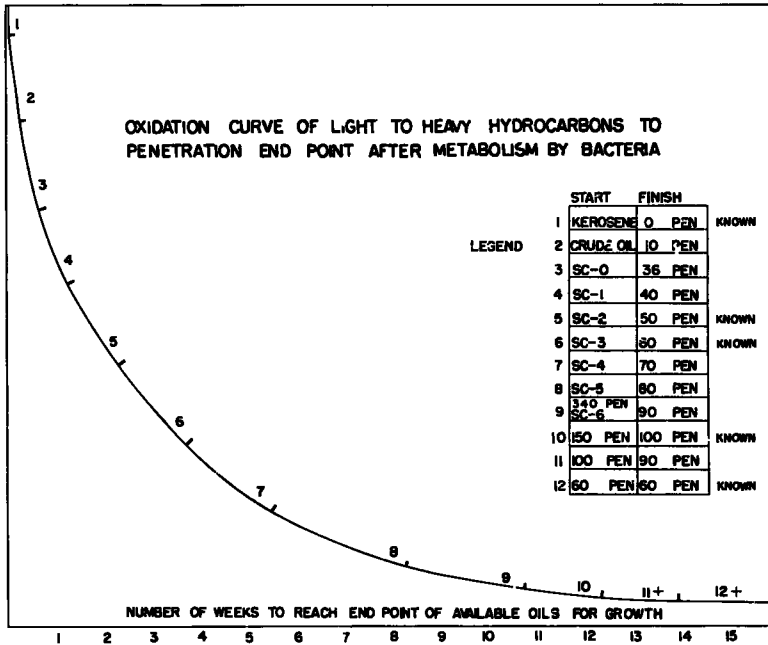NUMBER OF WEEKS TO REACH END POINT OF AVAILABLE OILS FOR GROWTH

Figure 9

form film. Otherwise the results will not correlate closely.

Temperature is vital to correlating test data. Whatever is found to be the easiest
constant temperature to maintain should be used, such as 78 F.; 100F. will accelerate
more growth. It is difficult to maintain sufficient moisture in the experiments, due to
evaporation at higher temperatures.

Since dispersion is one of the most-difficult problems of using heavy, viscous hydro-
carbons, such as asphalts, these materials are difficult to use in bacteriology labora-
tory experiments. However, in a properly oiled aggregate we find an ideal target for
microbial attack, because of the immense surface area of thin oil film presented on the
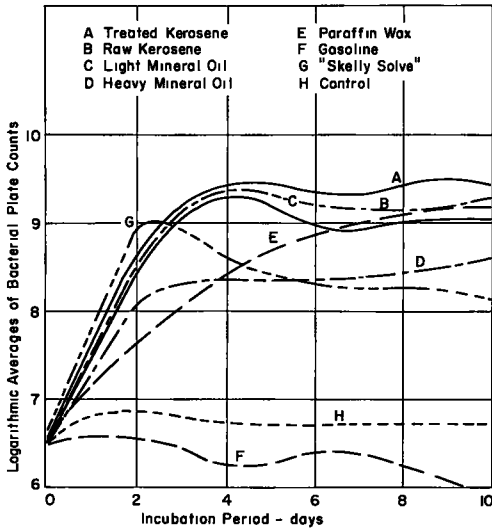bottom of the mat.

Once underway, this bacterial action migrates upward with capillary moisture as
the vehicle. As the oils of the asphalt binder are slowly oxidized, the asphalt becomes
progressively harder. The completed action leaves the aggregate with insufficient
binder (see Figures 2 and 3). Longitudinal cracks generally appear in the weakened
mat through the seal coat after ravelling starts on the bottom of the mat.

Aggregate thus freed from the mat is free to move and is no longer an integral part
of the mat. This outside edge of mat, having lost its vertical support, breaks and be-
comes an easy prey for encroaching traffic to thrust aside. This applies especially to
narrow mats. Access is thus gained for further oxidation. Not a very specticular
action nor one characterized by sudden results, but nature's methodical process of
changing a contaminant back to soil (see Figures 4, 5, 6, and 7).

It is not intended to imply that all the failures in old oil mat shoulders were the re-
sults of microbial attack, but, if conditions had been favorable to bacterial action, it
could be a contributing factor.

At present it is difficult to distinguish by casual observation the underlying cause of
shoulder failure, because so many factors are involved. So how much can be attribut-

ed to microorganisms remains to be discovered by careful sampling of failures and laboratory analysis of the extracted asphalt content remaining in the mat and examination of the base as a cause of the shoulder failure.

Figure 10. Growth of pseudomenas culture No. 8 on various hydrocarbons. This typical pseudomonas aeruginosa could not utilize gasoline like culture X. But this is significant in the laboratory only since nearly all strains and types are present in natural soils for the destruction of any hydrocarbon that might contaminate the soil.

SUMMARY BY BURGESS

Having established that certain types of asphalt mats are subject to bacterial attack at the shoulder, it is now logical to suggest a practical means to prevent this oxidation in future construction. Inhibiting the oxidation of road oils, used as a binder in asphalt mats, by soil bacteria could be successfully accomplished by undersealing the oil mat. A light $\frac{1}{8}$ inch membrane on the outer 18 inches of base before final laydown could provide an adequate underseal protection. This need not extend beyond the anticipated edge of the oil mat, but an extra width would provide additional protection for the base from runoff.

The membrane can be made of either hot-applied, 60-penetration, cat-blown asphalt or any 60-pen. asphalt of equal ductility at 30 F. or quick-breaking emulsion using 80-pen. base stock could be substituted, providing, it also had good or equal ductility.

The ultimate goal of this project is the elimination of shoulder maintenance due to this type of oxidation. Several maintenance foremen estimate 60 percent of oil-mat maintenance is shoulder replacement.

Syneresis or migration of the oil component of road oils as a factor contributing to the hardening of road oils is not compatible with known aggregate absorption as found in "Scoria" by the writer. This is not to be confused with evaporation of light ends or cutback from MC or RC road oils. But it is known that certain asphalts harden to some extent in tight containers. The cause of this is not clearly understood, although it is probably polymerization.

A high softening point (175 F.) is essential for an asphalt that is to be used for membrane.

The possibility of using chlorinated asphalt to inhibit bacterial activity is being explored.

INVESTIGATION BY STONE, FENSKE AND WHITE

The conditions necessary for attack on oils by microorganisms have been summarized by Tausson (1928) as follows: (1) presence of water with mineral salts; (2) a nitrogen source, such as the ammonium or nitrate ion; (3) free access of oxygen; (4) a neutral reaction and a buffer such as $CaCO_3$ to maintain it.

However, there has been little attention given as to what hydrocarbons are most subject to attack and the mechanism of their breakdown, although Tauson and co-workers (1934) have shown that some acids and unsaturation are produced in the bacterial dissimilation of crude and lubricating oils.

The purpose of this work was to determine how wide a range of petroleum actions could be readily attacked, to attempt to find which of several representative petroleum fractions were most subject to attack, and to isolate and characterize a number of organisms able to develop on a hydrocarbon medium.

DEVELOPMENT OF CULTURES ON OIL

Erlenmeyer flasks were prepared containing 0.5 g. oil, 50 ml. H_2O, 0.25 g. $CACO_3$, 0.25 percent NH_4NO_3, 0.1 percent Na_2HPO_4, 0.05 percent KH_2PO_4, 0.05 percent $MgSO_4$, 0.02 percent $MnCl_2$, and traces of Ca, Fe and Zn. The flasks were inoculated with one gram of garden soil, incubated for a period of from 10 to 20 days and shaken twice daily. When considerable decomposition appeared as indicated by the emulsification of the oil and increased turbidity of the medium, 1 ml. of the mixture was transferred to another flask containing all the above ingredients except soil.

After two to three transfers the breakdown of the oil proceeded faster and the period of incubation was shortened accordingly. Flasks were incubated at 20 deg. room temperature (23-26 deg.), 30 and 37 deg. C.

After at least six successive transfers from the original flask containing soil inoculum, bacterial counts were made on the medium by plating on standard nutrient agar. A mineral-salt oil agar was also used, but it was observed that nutrient agar gave slightly higher counts. Furthermore, in all cases tested the organisms which appeared on the mineral-salt oil medium grew when transplanted to the nutrient agar. As continued cultivation on nutrient agar caused a marked decrease in the ability of the cultures to attack hydrocarbons, cultures were picked from the plates in proportion to the numbers present and kept on the mineral-salt oil medium.

The materials used in this study included several crude oils, heavy oil residues such as petrolatum (Paraffinic) and asphaltic tar (aromatic) and the various oils given in Table 1. The filtered 185 Pennsylvania neutral was a conventionally refined 10-W grade of motor oil. This oil is typical of light oils or neutral made in the Pennsylvania area, and is a relatively homogeneous mixture of hydrocarbons. The distillation fractions of this oil, designated as Oils 2, 3, and 4 in Table 1, were prepared by high vacuum fractional distillation and differ from each other essentially in molecular weight. Each oil is a narrow boiling fraction with a very small molecular weight range.

Oils 5, 6, and 7 of Table 1 were prepared by solvent-extraction of oil with acetone (Hersh 1938). Thus, these fractions represent hydrocarbons differing essentially in molecular structure, whereas those prepared by distillation represent fractions differing essentially in molecular size or weight.

Oil 8 is a typical heavy grade oil with a higher molecular weight and more heterogeneous composition than Oil 1. Oil 9 resulted from exhaustive acetone extraction of Oil 8 and may be considered to be a very high molecular weight, highly paraffinic hydrocarbon mixture.

Figure 11. Growth of pseudomenas culture X. on various hydrocarbons. This culture which was isolated from water taken from the bottom of distillate storage tank had the ability to utilize ''Skelly-Solve'' which is a hydrocarbon fraction containing a high percentage of hexane, which was readily utilized, but cyclic compounds such as beneze and light mineral oil were not utilized.

Oil 10, an aromatic extract from a Louisiana light distillate oil, represents a relatively low molecular weight aromatic-type hydrocarbon oil.

Mixed cultures are developed from soil capable of attacking each oil or oil fraction studied as well as the crudes, petrolatum and asphalt. After several transfers the medium weight and crude oils were found to be quite rapidly broken down and usually com-
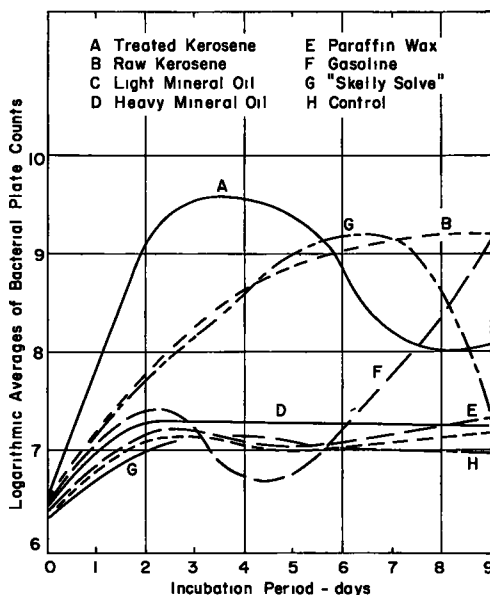
pletely emulsified in three to five days at room temperature. The heavier oils were more slowly dissimilated, the emulsification process requiring as long as three weeks. Bacterial counts were made on the various fermentations after six to ten consecutive transfers. Petrolatum and asphalt supported the growth of large numbers of organisms concentrated at the oil-water interface of the separate globules, but owing to the difficulties of dispersion, no counts were made on these materials.

Typical counts of the mixed flora found in the breakdown of crude oil and certain fractions are shown in Table 2. In the majority of cases the peak in bacterial flora occurred between the third and sixth day of fermentation. At this time there was usually one type of colony predominating in each type of oil. All the organisms examined from the various oils at the different temperatures appeared to be short, motile, gram-negative rods. In the crude oils, pseudomonas seemed to be the outstanding type. White opaque colonies appeared most frequently in the light oils. White and mucoid colonies were predominant in many of the heavy oils.

In most cases pigmented forms other than pseudomonas did not appear until after a peak in total count was reached. There was little indication of the specificity of a certain organism for a particular type of oil. However, if a mixed culture from a rapidly fermenting oil of highly aromatic nature was transferred to a paraffinic type (or vice versa) the growth lagged for several days. The culture was usually able to adapt itself to its environment and become active in the new oil within one transfer.

It was apparent that the lighter weight oils were more easily attacked than the heavier fractions. This is evident from the counts given in Table 2, if counts of Oils 2 and 4 are compared, or of Oils 2 and 8, or Oils 8 and 9. In the fermentation of mixtures containing as much as five to ten percent of Pennsylvania 185 neutral oil measurements of the unfermented oil showed an increase in viscosity, indicating a preference of the organisms for the lighter fractions. Also, it could be readily observed that the light oils disappeared from the surface of the medium much more quickly than the heavy ones. The crudes supported growth in a degree comparable to the light oils and were subject to rapid emulsification although globules of the heavy fractions could still be discerned on the surface after two weeks' fermentation.

The bacterial counts also indicate that the paraffinic oils were attacked more rapidly than the aromatic types as can be seen by comparing aromatic oils with paraffinic Oil 7. Oil 6, which contains a relatively high percent of naphthene compounds occupied an intermediate position with respect to growth supported. The slower attack of aromatic oils can also be shown by comparing Oil 10 (strongly aromatic) with Oil 7 or the light neutrals 1, 2, and 3. Although Oil 10 is more viscous than the light oils it has a smaller average molecular weight.

MANOMETRIC STUDIES

Several representative oils were chosen for determination of the oxygen required for their dissimilation by mixed cultures. The studies were carried out with a Warburg manometric apparatus immersed in a constant temperature bath and shaken continously by motor. The technique and apparatus used was similar to that described by Dixon (1934). The cultures were inoculated and cultivated in standard manometric flasks and respiration measured over a period of several days at 30 C. In each flask was placed 3.8 ml. of mineral salt solution, 50 mg. of the desired oil and 0.2 ml. of the corresponding mixed culture inoculated. The mineral salt solution was of the same composition as that described previously except that $(NH_4)_2SO_4$ was substituted for NH_4NO_3. Readings were taken twice daily for intervals of two hours, these figures were plotted and from the area subtended by the curve the total respiration during the entire time was calculated.

When the fermentations were carried out for longer than seven days, a large fluctuation in results was noted. Allowing for small errors in technique and the relatively large variation to be expected from mixed culture studies, the results obtained during the first five days of incubation were fairly consistent. In practically every case the peak of respiratory activity came in this interval. However, it was noted that the actual amounts of oxygen consumed and carbon dioxide liberated varied according to the rate

the temperature of incubation and the age of the inoculum. Some typical results are shown in Table 3. About 10 ml,of oxygen were taken up in the fermentation of Oils 1 and 7 containing predominatly paraffin hydrocarbons. In Oil 5 (aromatic) the uptake was slower. The breakdown of Oil 8, which has an average molecular weight of nearly two times the light oils and is more viscous, consumed only about half as much oxygen during the longer time interval. Oil 9 which is still heavier and composed of larger molecules had still less oxygen uptake.

As a check on the respiration to be expected from the cells alone, a control is shown based on 0.2 ml.of inoculum from oil fermentation suspended in buffer with no oil added. The amount of respiration is negligible and in comparison with the growing culture.

The carbon dioxide values are subject to an error as a small amount of gas would originate from the $CaCO_3$ buffer, not only by the action of organic acids that may have been formed during fermentation but also from free sulfuric acid produced by the utilization of nitrogen from the $(NH_4)_2SO_4$. The latter can be roughly estimated at between 0.08 and 0.1 ml.CO_2 for ml.of medium on an active culture producing nearly a gram of moist cells per liter, assuming that the cells contained 2.5 percent nitrogen on a wet basis. As large scale fermentations carried out under similar conditions indicate that usually about 80 percent of the original oil can be recovered, only about 0.20 to 0.30 ml. of CO_2 could be liberated by mono-carboxylic acids formed from 0.050 g. of the oil hav-

Figure 12

ing a ml.wt.in the range of 400. The total correction amounts to an approximate reduction of only 5 percent for the CO_2 evolution of the light oils. The heavy oils have been corrected accordingly.

The CO_2/O_2 ratios using the corrected values are subject to the errors present in the CO_2 determination but show fairly consistent values for the different oils. It is noteworthy that in the case of the light oils, the ratios are high for incomplete oxidation of the hydrocarbons. The theoretical respiratory quotient for complete oxidation of a long-chain paraffin hydrocarbons with the formula $CH_3(CH_2)_nCH_3$ is approximately 0.67.

$$(CH_2)_n + 1.5nO_2 -- nCO_2 + nH_2O$$
$$CO_2/O_2 - n/1.5 X n - 0.67$$
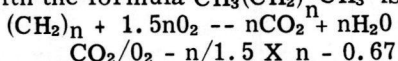
The respiratory quotients of the light oils are in the neighborhood of 0.65.

If the hydrocarbon molecules were oxidized without decarboxylation considerable loss of CO_2 the gas ratios should be much lower. The rest indicate that a large percentage of the molecules attacked were completely oxidized to CO_2.

The fermentation of the heavier oils containing longer molecules not only did not produce as much CO_2 but furthermore gave a much lower CO_2 to O_2 ratio. In the case of Oil 9 the CO_2 evolution was practically negligible, indicating a much less complete oxidation of the heavy oils.

COMMENT

It is evident from the studies presented here together with those of other investigators, that under favorable conditions microorganisms can be found that are capable of attacking practically any hydrocarbon from methane up to the heaviest paraffinic or asphaltic residues. However, the heavier oils become more difficult to attack as the viscosity and molecular weight increase. This is due in part to the fact that the more viscous oils are harder to disperse in a liquid medium and hence there is less surface exposed to the growth of microorganisms. But the difficulty of attack is probably also attributable to the larger molecule. Strawinski and Stone (1940) have found that compound the range of 10 or 16 carbon atoms are attacked more readily than those smaller molecular weight.

Observations by the authors as well as Bushnell and Haas (1940) on the fermentation of gasoline and kerosene indicate that, although both substances are quickly attacked, the kerosene is more subject to breakdown than the gasoline with bacterial counts up to a billion per ml. or comparable to counts observed in light oils. From these results, it is evident that in oils of a predominatly paraffinic nature the fractions from kerosene up to medium weight lubricating oils include the range most easily attacked by bacteria.

Both bacterial counts and manometric studies indicate that paraffinic oils are more easily broken down than corresponding oils of aromatic nature. However, it must be emphasized that the predominatly aromatic fractions proved to be very acceptable carbon and energy sources. The most unexpected observation in this connection was the apparent lack of specificity of one culture for any certain type of oil. Even when such studies are extended to pure hydrocarbons it is possible to find that an organism which is adapted to grow on a purely paraffinic source, such as cetane, will begin to grow immediately when transferred to a strictly aromatic compound such as naphthalene.

The organisms capable of attacking oils appear to be present wherever samples were taken. Only gram-negative rods were found, whether the enrichment cultures were incubated at 20C., 30 C., or 37 C. These findings are not entirely in accord with Solngen (1913) who observed mycobacteria at 37 C. nor with Bushnell and Haas (1941). One reason for the absence of acid-fast bacteria in the present work is that the cultures were transferred at intervals of 7 to 14 days and probably there was not sufficient time for the development of these slower-growing organisms.

It is evident, particularly among the non-pigmented forms, that there is no sharp line of demarcation between the different groups. The adaptability of these bacteria is further realized when it is considered that in most soil samples there is little chance of long chain hydrocarbons having been present for unnumbered bacterial generations. It is obvious that there is no specialized group of organisms here but that we are dealing with the common soil forms which possess the ability to adapt themselves to an infinite variety of organic compounds.

SUMMARY BY STONE, FENSKE AND WHITE

1. Cultures capable of attacking crude oil, lubricating oils, vaseline, asphalt, and all other petroleum fractions used were obtained from garden soil.

2. It was found that the light to medium-weight fractions are more subject to attack than the heavy viscous portions and that the paraffinic fractions are more readily broken down than the aromatic types.

3. The breakdown of oil is an oxidative change characterized by a high bacterial

count, emulsification and sometimes a decrease in pH.

4. There was much less oxygen uptake in fermentations of heavy oils compared to similar lighter fractions. Likewise aromatic fractions utilized less oxygen than the paraffinic types.

5. The CO_2 to O_2 ratio for the dissimilation of light oils is in the neighborhood of 0.65. In heavy oils the ratio drops to a much lower figure.

6. The organisms were all motile gram-negative rods including Pseudomonas, and many white-mucoid types. They were obtained from all soil samples tested and appear to be of common occurrence.

7. The cultures did not exhibit a specific ability to attack one type of oil but rather a capacity to adapt themselves, according to conditions, to attack the particular oil that was present.

SUMMARY OF STUDY BY BUSHNELL AND H. F. HAAS

Cultures of organisms capable of using petroleum fractions such as "Skelly-solve," gasoline, kerosene, light and heavy mineral oils, and paraffin wax as the source of carbon and energy for their metabolism were isolated from various petroleum storage tanks; they were found to be good sources for the isolation of organisms of this type. Organisms possessing this ability are not necessarily confined to such habitats, since practically all the pseudomona cultures, regardless of their origin, were capable of utilizing kerosene. This was demonstrated by the fact that cultures isolated from various sources, such as abscesses, mastitis, water, and fecal matter of animals, were all able to utilize it.

Bacteria of other genera were also found capable of this activity, including certain species of the micrococci, corynebacteria, and Culture X exhibited a preference for paraffinic to the naphthenic or cyclic hydrocarbons. Many of the cultures were able to withstand as high as 10 to 15 transfers under kerosene without diminution in growth, thereby indicating that accessory growth factors are not needed, or that the organisms were able to synthesize these substances.

Respiration studies indicated that the hydrocarbons were oxidized largely to carbon dioxide and water. The respiratory quotients of various bacterial cultures on different hydrocarbons varied from 0.30 to 0.70. No direct correlation between the respiratory quotient and the nature of the hydrocarbon was observed.

Some evidence was obtained to show that long-chain organic acids and unsaturated hydrocarbons were formed during the bacterial decomposition of the hydrocarbon fractions. The formation of organic acids was indicated by small changes in the pH of the medium and by the increased ease of formation of emulsions of oil and water.

The bacterial production of unsaturated hydrocarbons was indicated by changes in the distillation temperatures of the kerosene. The boiling point of the last 20 percent of kerosene distilled was higher, probably indicating that polymers with a higher boiling point were formed from the unsaturated hydrocarbons during the process of distillation.

As a result of this investigation, it has been established that the bacterial utilization of hydrocarbons is a characteristic common to many types of microorganisms and that in nature this process probably occurs to a greater extent than is generally recognized. The oxidation of hydrocarbons was found to occur on simple media; in fact, ordinary well water at the bottom of a distillate tank was able to support a bacterial count of approximately 900,000 organisms per milliliter.

The respiration studies indicated that the oxidation of hydrocarbons is similar to the oxidation of other organic compounds and that such end products as carbon dioxide, water organic acids, and unsaturated hydrocarbons are produced.

LITERATURE SEARCH BY BITUMINOUS DIVISION
OF BUREAU OF RECLAMATION

A literature search was undertaken in conjunction with the soil burial tests to attempt to uncover work of any bearing on service life of bituminous materials used for buried mem-

branes which may be subjected to conditions of service favorable to attack by micro-organisms. Considerable literature was found descriptive of the attack of microorganisms on a large variety of petroleum derived hydrocarbons and related compounds, but very little specifically on attack upon asphalt. However, since even the most durable rocks, and everything else, slowly yield to the succession of diversified attacks by species of fungi, bacteria, yeasts, molds, and powerful enzymes produced by them, it was thought wise to investigate the degree of susceptibility of asphalt to microorganism attack.

The ability of microorganisms to utilize petroleum hydrocarbons and related materials has been recognized for a long time. For instance, as long ago as 1906, Rohn wrote concerning the utilization of paraffin by fungi. Again, in 1913, Sohngen recorded the utilization of paraffins by certain fungi.

In 1917, P. L. Gaines, wrote concerning the effect of paraffin on the accumulation of amonia and nitrates in the soil (J. Agr. Research, Vol.10: 355-364).

In 1928, P. H. H. Gray and H. G. Thornton, in "Soil Bacteria that Decompose Certain Aromatic Compounds," Zentr. Bakt. Parasitenk. Abt. II, 73: 74-96, say that bacteria which are widely distributed in soil are able to oxidize substances which are usually thought to be potent bactericides. When soil was teated with hexane benzene, toluene, xylene, naphthalene phenol, cresol, resorcinol, phlorglucinol, pseudocumene, mesitylene, cymene and pinene; these genera were capable of utilizing one or more of the above compounds; micrococcus, Mycobacterium, Bacterium, Bacillus and Spirillum.

Vo. O. Tauson, in "The Oxidation of Benzene Hydrocarbons by Bacteria." Planta, 7: 735-757 (Chem. Abs. Vol. 23: 3945, 1929) found a great variety of microorganisms able to utiliz hydrocarbons, and isolated three of the species. Bacterium naphthalinicus B. naphthalinic (liquefaciens) and Bacterium naphthalinicus B. (non-liquefaciens) which oxidized hydrocarbon

Zo Bell, Grant, and Haas observed the growth of water bacteria in the presence of 1 percent phenol and a like concentration of emulsified tri-cresol. (Zo Bell, C. E., Grant, C. W., and Haas, H. F., "Marine Microorganisms Which Oxidize Petroleum Hydrocarbons." Bul. Am. Assn., Petrol. Geol., Vol. 27: 1175 (1943).

The bacteria did very well despite the presence of these strong chemicals which are normally considered good bactericides. They also found marine bacteria capable of utilizing petroleum ether, gasoline, kerosene, lubricating oil, crude oils, petroleum, paraffin and microcrystalline waxes, mineral oil, methane, pentane, hexane decane, trimethylpentane, tetratriacontane, benzene, toluene, xylene, cyclohexane, anthracene, naphthalene, pyridine, natural rubber, isoprene, neoprene, and other synthetic rubbers. The aromatic and cyclic hydrocarbons were very slowly utilized.

They state that most of the bacteria oxidize hydrocarbons only in the presence of free oxygen although some of them can utilize nitrate as a hydrogen acceptor and possibly some of them can activate sulfate as a hydrogen acceptor which allows the bacteria to utilize these compounds as food. (In the latter case hydrogen sulphide would be produced which inhibits bacterial oxidation in concentrations exceeding 0.0001 ml. per liter and would have to be removed from the system in some way for disintegration to progress). They found Proactinomyces, Actinomyces, Pseudomonas, Microspora, Mycobacterium and possibly other genera are able to oxidize hydrocarbons.

CONCLUSION BY BITUMINOUS SECTION, BUREAU OF RECLAMATION

The ultimate effect of the attack of microorganisms should not be underrated. The most durable materials of construction and even the most resistant rocks eventually crumble before the successive attacks of soil bacteria and fungi, or the enzymes and decomposition products produced by them. This is the ordinary process of soil formation. However, the rate of attack on asphalt of the proper hardness is so slow as to be of negligible effect on membranes where the temperature does not exceed 70 F. for those on the order of $\frac{1}{4}$ inch thick as used in Bureau of Reclamation construction.

Writer's note: These bureau tests were run with above normal ground temperatures in compost beds. Once again this points to the most important controlling factor; temperature. Moisture is important and a prerequisite, but the amount above minimum requirements is unimportant, likewise, oxygen and a favorable pH range. As temper-

atures decrease, bacterial activity slows until it becomes practically dormant at 50 F.

The natural temperature of the soil, four to six inches below the surface, is quite constant in the summer months and under these conditions the normal activity of soil bacteria and their catalytic enzymes is characterized by the orderly procedure found in the decomposition of dead organic material (asphalt not excluded) into plant nutrients and soil. But as the temperature is raised, the activity increases far beyond the normal rate (see Figure 12).

Figure 2 depicts new construction with conventional oil mat with top seal coat on a select borrow base course. Under normal conditions this base course is comparatively free of any soil bacteria because, very little, if any surface soil is present since the top soil with the overburden is removed in a new pit. Therefore, new construction is made with comparatively sterile soil which would remain sterile if protected from the migration of soil bacteria.

Figure 3 shows runoff percolating into the base at the interface of the mat with the base. Under normal conditions it takes two or three years for soil bacteria to slowly migrate across the borrow pit to the edge of the mat. This interval of time is sufficient to furnish the necessary moisture under the edge of the mat for the propogation of the hydrocarbon oxidizers which have migrated there.

Figure 4 demonstrates initial ravelling on the underside of the mat as the bacterial enzymes attack the oils in the asphalt. Ravelling on the bottom of the mat is a slow insiduous process involving each particle of the aggregate individually. The thin film of asphalt surrounding and covering large and small particles is very thin, especially in open graded aggregates, and the thinner the film the more vulnerable it is. It is fortunate indeed that mat temperatures are well below the minimum for the growth of bacteria 90% of the time. Otherwise the process of oxidation on road oils would be very rapid; such as found in the tropics on native resins used for soil binder (Jones, London University 1954). Even the most durable asphalts used for protective membranes are not immune to bacterial attack when a constant temperature of 104 F is maintained with hydrocarbon oxidizing bacteria present; such as found in the methane gas towers in a sewage disposal system in Koln Germany. This asphalt was of high quality with a softening point of 185 F. It had a thickness of one-fifth of an inch and in five months was practically useless as a protective membrane for the steel as seen by Picture No. 5.

Figure 5 shows the seal coat cracking which allows the first pieces to break away from the edge of the mat, which in turn allows moisture and oxygen to seep farther under the mat. The loss of vertical support coupled with a loss of strength makes the edge of the mat an easy prey to break down with traffic.

Figure 6 demonstrates the progressive ravelling caused by further inroads of oxidation on the bottom of the mat. This ravelled portion of the oil mat should be taken into consideration when determining asphalt content because this ravelled aggregate is generally compacted into the softened base where it loses its former identity.

Figure 7 depicts new construction which is designed to prevent bacterial attack by the use of an asphalt membrane, and also provides a happy medium to protect the base from runoff at the edge of the mat. It is highly probable, in the not too distant future, that considerable new construction will embody the use of asphalt membrane for enveloping the entire base either for retaining a prescribed amount of moisture in the base or protecting the base from excessive moisture and alleviation of frost boil damage by preventing ice lenses forming in the base from underground springs.

Figure 12 showing oxidation of asphalt membrane lining, as the result of bacterial attack on the interior of steel tank used for production of methane gas from sewage. This extreme oxidation is the result of preheating the sewage with steam to 104 F., which accelerates the activity of the bacteria. The membrane was approximately $3/16$ inch thick and a softening point of 185 F. The bubbles in the picture are the result of gas production in the asphalt proper from pinpoint intrusion or residual bacterial enzymes left on the surface after cleaning the tanks. Three weeks after this picture was taken, the asphalt was completely oxidized. It is highly probable that a highly chlorinated asphalt would resist oxidation even when subjected to these extreme conditions. It is unlikely that comparable results would be found in an asphalt mat where temperatures are at a minimum. Were it not for the enzymes-

catalyzed chemical reactions which help the bacterial utilization of hydrocarbons for energy or cell structure material, asphalt oil fractions would be comparatively immune to attack at normal temperatures.