The Production of Gum by Certain Species of Rhizobium

BY DEAN A. ANDERSON

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FARM CROPS AND SOILS
SOILS SUBSECTION

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The Production of Gum by Certain Species of Rhizobium*

BY DEAN A. ANDERSON**

It has long been known that the legume root-nodule bacteria produce a bacterial gum, but knowledge of the exact role of gum production in the metabolism of the organism is still rather incomplete. The results secured by a number of investigators indicate that gum production may be intimately connected in some way with the mechanism of symbiotic nitrogen fixation. In addition, some studies have shown that considerable differences exist in the quantity and chemical nature of the gum produced by different species of Rhizobium.

Gum production studies, therefore, may throw some light on the mechanism of nitrogen fixation and may also provide a method for classifying the various species of Rhizobium. Furthermore, the study of gum production by Rhizobium may yield results of considerable general scientific interest because of the widespread nature of gum production by microorganisms.

The work reported in this bulletin was planned to study the production of gum by various species of Rhizobium under different environmental conditions and to determine whether or not gum production would serve as a means of species differentiation.

REVIEW OF LITERATURE

Very little work has been done which deals directly with gum production by Rhizobium. In many studies of the physiology of the root-nodule bacteria, however, incidental observations have been made on gum production. Studies of the gums produced by other bacteria have also yielded some information which is of value in interpreting the results obtained in investigations of gum production by Rhizobium.

Several of the early investigators, such as Beijerinck (7), Prazmowski (43), Burrage (9), Harrison and Barlow (24, 25) and others, report the production of a characteristically slimy type of colony by the legume root-nodule bacteria.

Greig-Smith (21) claims that the nature of the slime "capsule" surrounding the organisms is influenced by the sugar source, glucose producing a "delicate membrane" while the capsule in sucrose solution is very tough.

* Part of a thesis submitted to the faculty of Iowa State College in partial fulfillment of the requirements for the degree doctor of philosophy. The work was conducted under project No. 266 of the Iowa Agricultural Experiment Station.

** The author wishes to express his appreciation to Dr. P. E. Brown and to Dr. R. H. Walker for their helpful suggestions in conducting the work and in the preparation of the manuscript.
Buchanan (8) made a study of gum production in 300 different nutrient solutions. Fifteen carbohydrates, representing pentoses, hexoses, bioses and trioses, were found favorable for gum production. Glycerol, some common glucosides and certain organic salts also favored gum production. Organisms from different legumes were found to differ in their ability to produce gums under identical conditions.

Gage (19) by qualitative comparisons found that rapid gum production occurred in cultures containing mannitol, dextrose, dulcitol and maltose. Gum development was slow in solutions containing galactose, sorbitol and sinistrin. No gum was found in mannan, pentosan and leichinin solutions. He isolated the gum by precipitation with alcohol and found that it was nitrogen free.

Greig-Smith (22) found that saccharose, levulose, maltose, mannitol and glycerol allowed a heavy production of gum by Rhizobium while lactose was unsuitable. The most suitable nitrogen sources were found to be asparagin and nitrates.

Several workers (3, 5, 10, 14, 18, 39, 44, 47) in their studies of the cultural characteristics of various strains and species of Rhizobium have referred either directly or indirectly to the amount and character of slime or gum produced by the organisms when grown on various media. None of these workers, however, made direct measurements of the amount or kind of gum produced.

The fact that Rhizobium in symbiosis with the host plant has the power of fixing atmospheric nitrogen and that it produces considerable quantities of gum has led to the conjecture that some relationship may exist between gum production and nitrogen fixation.

In 1898 Mazé (40) found large amounts of slime in cultures where appreciable amounts of nitrogen were fixed. He therefore concluded that the slime is a nitrogenous substance elaborated by the root-nodule bacteria.

Greig-Smith (22) concluded that Rhizobium is capable of fixing atmospheric nitrogen in the absence of the host plant “under conditions that favour slime production.”

Fred (17) found no relation between the amount of nitrogen fixed in solution cultures and the amount of gum produced.

The results which have been cited fail to show any relationship between gum production and nitrogen fixation in the absence of the host plant if such fixation occurs. The results obtained by other investigators, however, indicate that some relationship may exist between the ability of the organism to aid in symbiotic nitrogen fixation and its gum producing power.

Fred, Whiting and Hastings (18) point out that in general the production of gum seems to correlate with the ability of
Rhizobium to fix nitrogen in symbiosis with the host plant. This conclusion was drawn from the work of Stevens (45) and Wright (49), who found that certain strains of *Rh. meliloti* and *Rh. japonicum* which were efficient nitrogen fixers in symbiosis with the host produced relatively large quantities of gum. Inefficient strains of the same species were characterized by a moderately abundant to thin growth with a meager production of gum.

Thornton (46) found that the organisms apparently produce considerable slime just prior to their entrance into the root hairs of the host plant. He concluded that this slime production by the organism resulted from stimulation by certain substances secreted by the plant.

Buchanan (8), Greig-Smith (22) and Fred (17) isolated the gum produced by Rhizobium and showed that it contained no combined nitrogen. Buchanan also showed that the gum solutions did not reduce Fehling's solution, but on heating with acid in the autoclave at 15 pounds pressure for an hour hydrolysis occurred with the production of a reducing substance.

Greig-Smith (22) found the gum to be dextro-rotatory and on hydrolysis yielded dextrose and galactose, the former predominating.

Kramar (36) concluded that gum produced by Rhizobium is without question a carbohydrate yielding glucose on hydrolysis.

Hopkins, Peterson and Fred (32, 33, 34, 35) report the results of some intensive studies of the chemical nature of the gum produced by Rhizobium. Analyses showed that the cells contained, on the ash-free basis, 53.8 percent carbon and 4.69 percent nitrogen. The gum, on the same basis, contained 38.8 percent carbon and no nitrogen.

They determined the pentosan, uronic acid and reducing sugar content of the gums from *Rh. meliloti*, *Rh. trifolii* and *Rh. leguminosarum*. The most consistent difference between the chemical composition of the gums produced by the different species appeared in the content of uronic acid. The gum produced by *Rh. meliloti* contained only 4.1 to 6.8 percent uronic acid, while gum produced by *Rh. trifolii* contained 22.1 to 25.3 percent, and that produced by *Rh. leguminosarum* contained 19.3 to 22.0 percent. These results are of particular interest in connection with certain results secured in the present investigation.

Upon hydrolysis, the gums yielded from 70 to 75 percent reducing sugar, which was shown by saccharimeter readings, melting point of the osazone and fermentation tests to be glucose.

The gums produced by different species of Rhizobium are apparently different in composition. A considerable variation appears in the uronic acid and pentosan content of the gum from *Rh. meliloti* compared with the other gums. The gums from *Rh.
trifolii and Rh. leguminosarum were found to be similar in uronic acid and pentosan content, but differed in reducing sugar content, fermentation reactions and crystallization.

In recent years the chemical composition of several gums from different sources has been investigated rather carefully. The analyses indicate that gums are rather similar in their general chemical constitution, differing chiefly in the nature of the units which make up the gum complex. The gum produced by Rhizobium is similar in many respects to other bacterial gums.

Norman (42) in a study of the products of hydrolysis of gum arabic came to the conclusion that gum arabic is not a substance of definite empirical formula. “It is possible, however, to indicate its general composition—a nucleus acid consisting of galactose and a uronic acid, probably galacturonic acid, to which is linked arabinose by glucosidic linkages. The arabinose is in consequence more easily split off than the other components.”

Candlin and Schryver (12) suggest the use of the term “polyuronides” to describe substances made up of sugars linked to uronic acid molecules. The gums would, on this basis, be classed as polyuronides.

Cretcher and Butler (13) report that an aldobionic acid is obtained on the hydrolysis of gum arabic with acid. They later conclude (11) that the aldobionic acid from gum arabic consists of galactose and glucuronic acid. To this nucleus are attached molecules of galactose, arabinose and rhamnose.

Heidelberger and his associates (26, 28) published a series of papers on the chemical nature of the gums or “specific polysaccharides” produced by the various types of Pneumococcus. Capsular material was isolated from cultures of Type II Pneumococcus and was shown to be a carbohydrate built up of glucose molecules. The polysaccharide was thought to be the specific substance produced by the organism.

In another paper of the series, they (29) reported that the specific properties of the polysaccharide disappear when it is hydrolyzed. The products of hydrolysis are glucose and an aldobionic acid composed of a glucose and a glucuronic acid molecule.

In a later paper (30) they demonstrate that the uronic acid of the Type III Pneumococcus polysaccharide is glucuronic acid.

In another series of papers (4, 27, 20), they conclude that the polysaccharide produced by Friedländer's bacillus contains an aldobionic acid nucleus and is similar in some other respects to that produced by Pneumococcus.

Hamilton (23) recently concluded that the gum produced by Azotobacter chroococcum is a carbohydrate “of the higher series.” Boiling with acid failed to give it reducing properties. It was shown to be laevo-rotatory. A trace of nitrogen was found in the gum but it was attributed to impurities.
EXPERIMENTAL

The purposes of the present investigation were: (a) to develop a rapid method for measuring gum production by Rhizobium, (b) to compare gum production by different species of Rhizobium, (c) to study the rate of gum production, (d) to test the influence of environmental factors on gum production and (e) to learn something of the role of gum production in the metabolism of the organism.

The cultures of Rhizobium used in the investigation were secured from the laboratory collection. In order to eliminate errors due to impure cultures, all of the cultures were tested for purity in milk according to the procedure of Löhnis and Hansen (39). Some of the cultures have been carried in the laboratory for several years while others were isolated rather recently. This difference in the history, however, apparently did not influence the characteristics of the organisms. Most of the cultures were tested and found to produce nodules on the host plant.

The numbers used throughout the experiment for designating the cultures and the species or cross-inoculation groups to which they belong are listed below:

<table>
<thead>
<tr>
<th>Species or cross-inoculation group</th>
<th>Laboratory numbers used to designate the cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rhizobium meliloti</em></td>
<td>101-133</td>
</tr>
<tr>
<td><em>Rhizobium trifolii</em></td>
<td>201-208</td>
</tr>
<tr>
<td><em>Rhizobium leguminosarum</em></td>
<td>301-306</td>
</tr>
<tr>
<td><em>Rhizobium japonicum</em></td>
<td>401-416</td>
</tr>
<tr>
<td><em>Rhizobium phaseoli</em></td>
<td>501-503</td>
</tr>
<tr>
<td>Cowpea group</td>
<td>603-609</td>
</tr>
<tr>
<td>Dalea group</td>
<td>701</td>
</tr>
<tr>
<td><em>Rhizobium lupini</em></td>
<td>801-803</td>
</tr>
</tbody>
</table>

THE GRAVIMETRIC METHOD

The first problem was to develop some satisfactory method for determining the amount of gum present in solution cultures of Rhizobium. The method which has been used in the past consists of precipitating the gum by adding acetone or 95 percent alcohol and then filtering, drying and weighing the precipitate.

In preliminary experiments these two precipitants were compared, the effect of calcium carbonate on gum production was studied, and a satisfactory technique for filtering the gum was sought.

The results of these tests show that acetone and alcohol were equally effective as precipitating agents, but the gum precipitated with acetone formed into larger clumps and was therefore somewhat easier to filter; calcium carbonate stimulated gum production, as organisms grown in the medium containing
calcium carbonate produced nearly twice as much gum on the ash-free basis as those grown in the medium containing calcium chloride; and the use of a Gooch crucible for filtering the precipitated gum was found impracticable because of the extreme slowness of filtration. The most satisfactory results were obtained by using a tared filter paper placed in the bottom of a Hirsch funnel in such a manner as to form a shallow cup. For the drying and weighing operations the filter paper was placed in aluminum weighing boxes. The gum was dried at about 90 degrees C.

THE VISCOSITY METHOD

Preliminary experiments indicated that the gravimetric method of estimating the amount of gum produced by Rhizobium was extremely slow and that a more rapid method would be very desirable.

Walker (48), following the procedure developed by Levine and his coworkers (37, 38) used an Ostwald viscosimeter for measuring the gelatin liquefying power of Rhizobium. The possibility of using the same instrument for estimating the gum content of Rhizobium cultures, therefore, suggested itself. The assumption was made that, since the gum produced by Rhizobium is an hydrophilic colloid, it might serve to increase the viscosity of the culture medium. It was further assumed that if the viscosity increase was found to be proportional to the amount of gum present, viscosity measurements could be used as a means of estimating the amount of gum production.

A survey of the literature failed to reveal any previous investigations dealing with the utilization of viscosity determinations as a direct measure of the gum content of bacterial cultures. Falk and Harrison (16) and Falk (15) studied the effect of hydrogen ion concentration on the viscosity of bacterial suspensions, but no details of the method used in measuring viscosity were given.

A preliminary test was made to determine whether or not Rhizobium produces any measureable increase in the viscosity of a culture medium. For the test a 6-day culture of *Rhizobium leguminosarum* (305) in a yeast-extract-mannitol medium was used. The relative viscosity of the solution was determined with an Ostwald viscosimeter. Thirty-eight seconds were required to deliver 2 cc. of water at a temperature of 23 degrees C. in this viscosimeter while 87 seconds were required to deliver a similar amount of the Rhizobium culture. It is apparent that considerable viscosity may be developed by Rhizobium cultures.

THE RELATION OF VISCOSITY TO THE AMOUNT OF GUM PRESENT IN SOLUTION CULTURES OF RHIZOBIUM

In this test several cultures were used and the composition of the medium was as follows:
Glucose ___________________ 10.0 gm.
K₂HPO₄ ___________________ 0.5 gm.
MgSO₄·7H₂O _______________ 0.2 gm.
CaCl₂·4H₂O ________________ 0.1 gm.
NaCl ______________________ 0.1 gm.
Yeast-extract* _____________ 1.0 gm.
Distilled water _____________ 1,000 cc.
Reaction — pH 6.8

One hundred cc. portions of the above medium were placed in 500 cc. Erlenmeyer flasks, sterilized at 250 degrees C. for 18 minutes and duplicate cultures prepared, using 1 cc. of a 72-hour solution culture and incubating for 8 days at 28 degrees C. At the end of this period the culture was shaken for 1 minute, to give a uniform suspension. A 5 cc. sample was then pipetted into the Ostwald viscosimeter and the viscosity determined.

In order to control the temperature the viscosimeter was immersed in a Freas constant temperature water bath at a temperature of 30 ± 0.5 degrees C. The length of time necessary for the solution to flow out of the viscosimeter bulb was measured with a stop watch.

After each viscosity determination the viscosimeter was carefully washed with distilled water. In order to determine whether or not the removal of the bacterial gum from the viscosimeter was complete, the viscosity of water was measured occasionally in the viscosimeter during each experiment.

Immediately after the viscosity of each culture was determined, the gum in the remaining portion of the culture was pre-

**TABLE I. RELATION OF THE VISCOSITY OF SOLUTION CULTURES OF RHIZOBIUM TO THE GUM CONTENT.**

<table>
<thead>
<tr>
<th>Culture No.</th>
<th>Time of flow in seconds</th>
<th>Milligrams of gum in 95 cc. of solution culture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rh. meliloti 130a</strong></td>
<td>39.0</td>
<td>48.0</td>
</tr>
<tr>
<td><strong>Rh. meliloti 130b</strong></td>
<td>39.2</td>
<td>51.9</td>
</tr>
<tr>
<td><strong>Rh. trifolii 203a</strong></td>
<td>53.0</td>
<td>69.0</td>
</tr>
<tr>
<td><strong>Rh. trifolii 203b</strong></td>
<td>49.6</td>
<td>64.4</td>
</tr>
<tr>
<td><strong>Rh. leguminosarum 302a</strong></td>
<td>73.4</td>
<td>92.6</td>
</tr>
<tr>
<td><strong>Rh. leguminosarum 302b</strong></td>
<td>76.9</td>
<td>97.4</td>
</tr>
<tr>
<td><strong>Rh. leguminosarum 305a</strong></td>
<td>84.6</td>
<td>118.2</td>
</tr>
<tr>
<td><strong>Rh. leguminosarum 305b</strong></td>
<td>95.7</td>
<td>178.7</td>
</tr>
<tr>
<td><strong>Rh. japonicum 416a</strong></td>
<td>38.9</td>
<td>32.4</td>
</tr>
<tr>
<td><strong>Rh. japonicum 416b</strong></td>
<td>35.8</td>
<td>40.7</td>
</tr>
<tr>
<td><strong>Rh. phaseoli 501a</strong></td>
<td>50.6</td>
<td>67.3</td>
</tr>
<tr>
<td><strong>Rh. phaseoli 501b</strong></td>
<td>52.3</td>
<td>69.6</td>
</tr>
<tr>
<td>Cowpea bacteria 603a</td>
<td>40.1</td>
<td>*</td>
</tr>
<tr>
<td>Cowpea bacteria 603b</td>
<td>41.0</td>
<td>*</td>
</tr>
<tr>
<td><strong>Rh. lupini 802a</strong></td>
<td>38.8</td>
<td>43.4</td>
</tr>
<tr>
<td><strong>Rh. lupini 802b</strong></td>
<td>39.2</td>
<td>44.0</td>
</tr>
</tbody>
</table>

* Precipitate too fine to filter.
** Due to the large flakes of gum produced by this culture it was impossible to make an accurate viscosity determination.
cipitated by the addition of 95 percent alcohol, removed by filtration, dried and weighed. The viscosity of the culture was then compared with the amount of gum present.

The results of the experiment appear in table I. As may be noted, the time required for the solution to flow out of the viscosimeter bulb varied from 38.8 seconds with cultures 416b and 802a to 95.7 seconds with culture 305b. The time of flow of the sterile medium was 38.2 seconds.

The cultures fell into two groups—those which were just slightly more viscid than the control solution and those which showed a rather high degree of viscosity.

The amount of gum produced by the low viscosity cultures varied from 32.4 to 51.9 milligrams per culture. On the other hand, in the cultures showing high viscosity, the gum content ranged from 64.4 to 179.7 milligrams.

In order to determine the nature of the relationship existing between the viscosity of the cultures and their gum content, the data were analyzed graphically by plotting the viscosity read-

![Graph](image-url)

Fig. 1. The relationship between the viscosity of solution cultures of Rhizobium and the gum content of the solutions.
ings in seconds against the milligrams of gum in the cultures. The results appear in fig. 1.

The graph shows that in the low viscosity cultures, the viscosity did not bear a direct relationship to the gum content of the solution. With the cultures showing high viscosity, however, the viscosity was directly proportional to the amount of gum in the culture.

Certain of the Rhizobium cultures in this test failed to show the increase in viscosity which might be predicted from the amount of gum present, while in others a marked increase in viscosity was found which was proportional to the amount of gum present.

Two explanations of these differences appear: (a) In the low viscosity cultures the quantity of gum might have been insufficient to produce any viscosity increases, or (b) the chemical nature of the gum produced by the low viscosity organisms might have been such that it did not exert the same influence on viscosity as the gum produced by the organisms whose cultures showed high viscosity. The results of an incomplete experiment may be cited at this point because they throw some light on this question.

In this experiment the viscosity of a considerable number of cultures of Rhizobium was measured. The gum in the cultures was then precipitated with alcohol in order to determine gravimetrically the gum content. For most of the cultures, redistilled alcohol was used and precipitation was incomplete. As a result, these cultures were discarded. To the few remaining cultures 95 percent alcohol was added. The gum content of these few cultures was determined, and the data are tabulated below:

<table>
<thead>
<tr>
<th>Culture No.</th>
<th>Time of flow in seconds</th>
<th>Milligrams of gum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterile medium</td>
<td>42.6</td>
<td>73.6</td>
</tr>
<tr>
<td><em>Rh. meliloti</em> 133a</td>
<td>43.6</td>
<td>71.3</td>
</tr>
<tr>
<td><em>Rh. meliloti</em> 133b</td>
<td>44.1</td>
<td>81.5</td>
</tr>
<tr>
<td><em>Rh. leguminosarum</em> 302a</td>
<td>62.6</td>
<td>65.0</td>
</tr>
<tr>
<td><em>Rh. leguminosarum</em> 302b</td>
<td>53.8</td>
<td>92.0</td>
</tr>
<tr>
<td><em>Rh. leguminosarum</em> 306</td>
<td>69.8</td>
<td></td>
</tr>
</tbody>
</table>

Analysis of the data indicates that although cultures 133a and 133b contained more gum than culture 302b, they were much less viscid than the latter culture and the viscosities of cultures 302a, 302b and 306 were proportional to the amount of gum present.

On the basis of the data which have been presented it is evident that with species of Rhizobium whose cultures show high
viscosity, the viscosity measurements give a satisfactory estimate of the relative amounts of gum present in the cultures.

THE RELATION OF CONCENTRATION TO THE VISCOSITY OF GUM ARABIC SOLUTIONS

The experiments which have been cited indicate that the viscosity of solution cultures of certain species of Rhizobium is proportional to the amount of gum which these cultures contain.

In order to determine whether or not this same relationship is shown by other types of gums, an experiment was performed to test the effect of various concentrations of gum arabic on the viscosity of the gum arabic solutions. This gum was chosen because it is readily obtainable in a relatively pure form and is probably similar in chemical constitution to the gum produced by Rhizobium. The grade of gum used was U. S. Pharmacopoeia. It was a white powder which passed readily into solution.

Three tests were carried out as follows:

Test 1. A gum arabic solution was prepared by placing 1.0 gram of gum arabic into 100 cc. of distilled water. The solution was then set aside in the refrigerator for 24 hours. This allowed sufficient time for the solution to come to equilibrium, and the low temperature inhibited the growth of bacteria in the solution. The solution was then removed, carefully shaken and a series of dilutions prepared. The viscosities of the resultant solutions were determined at a temperature of 29.5 degrees C.

Test 2. The solutions were prepared by carefully weighing samples of gum arabic (beginning with 0.1 gram and increasing by tenths of a gram up to 1.0 gram) into 250 cc. Erlenmeyer flasks. One hundred cc. of distilled water were then added to each flask and the flasks placed in the refrigerator for 24 hours. Viscosity determinations were then made at 28 degrees C.

Test 3. Samples of gum were weighed out in the same manner as in test 2. Instead of dissolving the gum in distilled water, however, 100 cc. portions of glucose-yeast-extract medium were added to each of the flasks. The remainder of the procedure was the same as that followed in test 2.

The results of the first experiment appear in table II and those of the second and third tests are given in table III. These data show that the viscosity of the solution was more or less proportional to the concentration of gum. A remarkable difference, however, existed in the magnitude of the viscosity readings secured in test 2 and test 3. The only treatment dif-

<table>
<thead>
<tr>
<th>Grams of gum per 100 cc. of solution</th>
<th>Time of flow in seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>65.8</td>
</tr>
<tr>
<td>0.90</td>
<td>64.4</td>
</tr>
<tr>
<td>0.81</td>
<td>62.4</td>
</tr>
<tr>
<td>0.73</td>
<td>60.7</td>
</tr>
<tr>
<td>0.66</td>
<td>60.2</td>
</tr>
<tr>
<td>0.58</td>
<td>54.3</td>
</tr>
<tr>
<td>0.43</td>
<td>51.9</td>
</tr>
<tr>
<td>0.39</td>
<td>51.1</td>
</tr>
<tr>
<td>Water</td>
<td>40.4</td>
</tr>
</tbody>
</table>
TABLE III. RELATION OF THE CONCENTRATION OF GUM ARABIC TO THE VISCOSITY OF THE SOLUTION.
Comparative viscosity for the same concentrations dissolved in distilled water and in yeast-extract-glucose medium.

<table>
<thead>
<tr>
<th>Grams of gum per 100 cc. of solution</th>
<th>Time of flow in seconds. Solvent: water</th>
<th>Time of flow in seconds. Solvent: medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>70.2</td>
<td>52.6</td>
</tr>
<tr>
<td>0.9</td>
<td>67.4</td>
<td>51.3</td>
</tr>
<tr>
<td>0.8</td>
<td>64.6</td>
<td>49.9</td>
</tr>
<tr>
<td>0.7</td>
<td>61.7</td>
<td>48.4</td>
</tr>
<tr>
<td>0.6</td>
<td>59.5</td>
<td>47.2</td>
</tr>
<tr>
<td>0.5</td>
<td>58.7</td>
<td>46.3</td>
</tr>
<tr>
<td>0.4</td>
<td>54.8</td>
<td>45.1</td>
</tr>
<tr>
<td>0.3</td>
<td>51.4</td>
<td>44.1</td>
</tr>
<tr>
<td>0.2</td>
<td>47.9</td>
<td>43.1</td>
</tr>
<tr>
<td>0.1</td>
<td>44.5</td>
<td>42.1</td>
</tr>
<tr>
<td>Water</td>
<td>41.4</td>
<td></td>
</tr>
</tbody>
</table>

The results are shown graphically in fig. 2. The graph revealed a peculiar condition. In the water solution of the gum

![Graph](https://via.placeholder.com/150)

Fig. 2. The relationship between the concentration of gum and the viscosity of solutions of gum arabic.
the viscosity was practically proportional to the gum concentration until the concentration of gum exceeded 0.5 gram per 100 cc. A break in the curve then occurred between 0.5 and 0.8 gram. At concentrations between 0.8 gram and 1.0 gram the relationship again became proportional. In the glucose-yeast-extract gum solution the viscosity was nearly proportional to the gum concentration in all concentrations studied.

Although gum arabic solutions are not the same as bacterial cultures, the results secured with the gum arabic solutions are similar to those secured with certain cultures of Rhizobium. Therefore, the results of these two dissimilar tests are thought to justify the assumption that for certain species of Rhizobium, viscosity measurements offer a satisfactory means of determining the relative amount of bacterial gum in solution cultures.

EFFECT OF SHAKING ON THE VISCOSITY OF SOLUTION CULTURES

In the course of the development of the viscosity method it was thought that shaking might change the viscosity of the cultures. Bancroft (6) has pointed out that the viscosity of certain colloidal solutions of gelatin and other substances changes when they are shaken violently.

With this in mind an experiment was planned to determine the effect of shaking on viscosity. Seven-day-old cultures of Rhizobium were agitated gently for 1 minute in order to obtain a uniform suspension of gum and organisms. A 5 cc. sample was then withdrawn and the viscosity of the sample determined. After withdrawing the sample a rubber stopper was placed in the flask, the culture was shaken violently for 1 minute and the viscosity of a second sample determined. This procedure was repeated with two of the cultures until the culture had been shaken for a total of 5 minutes with viscosity determinations being made at the end of each minute. With the other cultures the shaking was continued after the first or second reading for a total of 5 minutes. Viscosity was then determined.

It is evident from the results given in table IV that shaking did not influence the viscosity of the cultures tested. This indicates that the gum in the Rhizobium cultures does not form a structure such as that shown by gelatin. Hence shaking did not change the viscosity of the culture solutions as it does with gelatin solutions according to Bancroft. In addition, the test indicates that the gum is probably an integral part of the solution rather than existing as a capsule around the organism. It may be thought that the initial shaking probably destroyed the gum structure. This point was repeatedly tested in subsequent experiments, however, and results showed that neither very gentle nor very violent shaking has any influence on the viscosity of the culture.
TABLE IV. EFFECT OF SHAKING ON THE VISCOSITY OF RHIZOBIUM CULTURES.

<table>
<thead>
<tr>
<th>Culture No.</th>
<th>Time of flow in seconds after shaking for various periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shaken 1 min.</td>
</tr>
<tr>
<td><em>Rh. meliloti 131</em></td>
<td>44.2</td>
</tr>
<tr>
<td><em>Rh. trifoli 206</em></td>
<td>119.6</td>
</tr>
<tr>
<td>Cowpea organism 603</td>
<td>48.7</td>
</tr>
<tr>
<td><em>Rh. lupini 803</em></td>
<td>42.9</td>
</tr>
</tbody>
</table>

* Cultures shaken continuously.

EFFECT OF THE ADDITION OF ACID OR ALKALI ON VISCOSITY

As another factor which might influence viscosity, the reaction of the medium was considered. A study of the effect of an addition of acid and of alkali on the viscosity of the bacterial cultures, therefore, seemed desirable.

For the test, 7-day-old cultures of strains 206, 305 and 501, all of which produce relatively high viscosity, were used. The cultures were shaken to give a uniform suspension and three 25 cc. portions were taken from each flask. One portion served as the untreated control; to the second, 0.25 cc. of 0.1 N HCl was added and to the third 0.25 cc. of 0.1 N NaOH was added. The cultures were then shaken and the viscosities determined.

The results of the test are given in table V. The figures show that the addition of acid or alkali produced no measureable effect on the viscosity of the cultures. The readings agree within the limits of accuracy of the viscosity test.

TABLE V. EFFECT OF THE ADDITION OF ACID OR ALKALI ON VISCOSITY OF SOLUTION CULTURES OF RHIZOBIUM.

<table>
<thead>
<tr>
<th>Culture No.</th>
<th>Time of flow in seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control solution</td>
</tr>
<tr>
<td><em>Rh. trifoli 206</em></td>
<td>81.2</td>
</tr>
<tr>
<td><em>Rh. leguminosarum 305</em></td>
<td>66.5</td>
</tr>
<tr>
<td><em>Rh. phaseoli 501</em></td>
<td>53.3</td>
</tr>
</tbody>
</table>

EFFECT OF SIZE OF INOCULUM ON THE VISCOSITY OF CULTURE SOLUTIONS OF RHIZOBIUM

In the results previously discussed it was noted that with certain species of Rhizobium a 7-day-old culture was fairly viscid while other species of Rhizobium developed practically no viscosity in the medium in the same period of time. In general it was found that the species of Rhizobium which produced viscid cultures were also characterized by rapid growth on solid
media. On the other hand, the species which produced little viscosity made a comparatively slow growth on solid media.

On the basis of this fact it was evident that the size of inoculum might have something to do with the viscosity. Furthermore, the question arose whether a heavy inoculation of a slow-growing organism would produce as large an amount of gum in the 7-day incubation period as a light inoculation of a rapid growing organism.

A test was made, therefore, to determine the effect of size of inoculum on the viscosity produced by various cultures.

The medium used was the yeast-extract-glucose solution prepared as previously described. Six flasks of sterile medium were prepared for each organism tested. This provided two triplicate series of cultures. A 72-hour culture of the organisms was used for inoculating the solutions. Three of the cultures in each series received 1 cc. of the inoculum while the other three cultures were seeded with 2 cc. of the same inoculum. The cultures were then incubated at 28 degrees C. for 7 days. On alternate days during this period, the cultures were removed from the incubator and shaken gently for approximately 20 seconds. This served to prevent the forming of clumps in the medium.

At the end of the incubation period the viscosity of each of the cultures was determined. The viscosimeter used, due to the

<table>
<thead>
<tr>
<th>TABLE VI. THE EFFECT OF SIZE OF INOCULUM ON THE VISCOSITY OF SOLUTION CULTURES OF RHIZOBIUM.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture No.</td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td>Sterile culture solution</td>
</tr>
<tr>
<td><em>Rh. trifolii 205</em></td>
</tr>
<tr>
<td><em>Rh. trifolii 205</em></td>
</tr>
<tr>
<td><em>Rh. trifolii 206</em></td>
</tr>
<tr>
<td><em>Rh. trifolii 206</em></td>
</tr>
<tr>
<td><em>Rh. leguminosarum 302</em></td>
</tr>
<tr>
<td><em>Rh. leguminosarum 302</em></td>
</tr>
<tr>
<td><em>Rh. leguminosarum 305</em></td>
</tr>
<tr>
<td><em>Rh. leguminosarum 305</em></td>
</tr>
<tr>
<td><em>Rh. japonicum 410</em></td>
</tr>
<tr>
<td><em>Rh. japonicum 410</em></td>
</tr>
<tr>
<td><em>Rh. japonicum 416</em></td>
</tr>
<tr>
<td><em>Rh. japonicum 416</em></td>
</tr>
<tr>
<td><em>Rh. phaseoli 501</em></td>
</tr>
<tr>
<td><em>Rh. phaseoli 501</em></td>
</tr>
<tr>
<td><em>Rh. phaseoli 508</em></td>
</tr>
<tr>
<td><em>Rh. phaseoli 508</em></td>
</tr>
</tbody>
</table>
smaller bore of its capillary, gave slightly higher readings than the one used in the first experiment.

The results of the test are summarized in table VI. An analysis of the data shows definitely that with all of the cultures tested, the size of inoculum used had no effect on the viscosity of the culture.

THE DAILY PRODUCTION OF GUM AS MEASURED BY INCREASES IN THE VISCOSITY OF SOLUTION CULTURES

Previous experiments have shown that a marked difference exists in the viscosity of solution cultures of Rhizobium after a 7-day incubation period. Nothing was known, however, as to the rate at which the viscosity increases may occur. A study of the daily viscosity increases of these organisms, therefore, seemed desirable.

In the course of the study two separate tests were made. Various cultures were used in the tests as shown in tables VII and VIII. The cultures were prepared by placing 100 cc. portions of yeast-extract-glucose medium in 500 cc. Erlenmeyer flasks, sterilizing, and inoculating with 1 cc. of a 72-hour culture.

TABLE VII. DAILY VISCOSITY MEASUREMENTS OF SOLUTION CULTURES OF RHIZOBIUM. TEST 1.

<table>
<thead>
<tr>
<th>Culture No.</th>
<th>Time of flow in seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Rh. meliloti 130</td>
<td>38.2</td>
</tr>
<tr>
<td>Rh. trifolii 203</td>
<td>39.1</td>
</tr>
<tr>
<td>Rh. leguminosarum 302</td>
<td>41.6</td>
</tr>
<tr>
<td>Rh. leguminosarum 305</td>
<td>41.6</td>
</tr>
<tr>
<td>Rh. japonicum 416</td>
<td>39.2</td>
</tr>
<tr>
<td>Rh. phaseoli 501</td>
<td>41.0</td>
</tr>
<tr>
<td>Cowpea organism 603</td>
<td>39.2</td>
</tr>
<tr>
<td>Rh. lupini 802</td>
<td>39.8</td>
</tr>
</tbody>
</table>

The viscosity measurements were made daily. The procedure consisted of shaking the culture by a circular motion for 1 minu­nute, then withdrawing a 5 cc. sample with a sterile pipette.

The viscosimeter used in the first test gave a time of flow of 37.6 seconds with water at 30 degrees C. Readings in this test were made at 30 degrees C. A different viscosimeter used in the second test gave a time of flow of 41.6 seconds with water at 28 degrees C. Readings on the viscosities of the cultures were made at this temperature in the second test.

The results of these tests appear in tables VII and VIII. An analysis of the data shows that in the first test the increase in viscosity of the high viscosity cultures, 203, 302, 305 and 501, was more or less regular during the entire period. The other cultures tested showed no appreciable increase in viscosity.
TABLE VIII. DAILY VISCOSITY MEASUREMENTS OF SOLUTION CULTURES OF RHIZOBIUM. TEST 2.

<table>
<thead>
<tr>
<th>Culture No.</th>
<th>Control</th>
<th>1 day</th>
<th>2 days</th>
<th>3 days</th>
<th>4 days</th>
<th>5 days</th>
<th>6 days</th>
<th>7 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>42.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rh. meliloti 130</td>
<td>42.7</td>
<td>43.2</td>
<td>43.1</td>
<td>42.9</td>
<td>42.7</td>
<td>43.5</td>
<td>42.7</td>
<td></td>
</tr>
<tr>
<td>Rh. meliloti 131</td>
<td>43.1</td>
<td>43.8</td>
<td>44.0</td>
<td>44.1</td>
<td>44.5</td>
<td>44.8</td>
<td>44.7</td>
<td></td>
</tr>
<tr>
<td>Rh. meliloti 133</td>
<td>42.6</td>
<td>43.4</td>
<td>43.4</td>
<td>43.4</td>
<td>43.6</td>
<td>43.8</td>
<td>43.8</td>
<td></td>
</tr>
<tr>
<td>Rh. trifolii 205</td>
<td>48.3</td>
<td>55.8</td>
<td>65.4</td>
<td>73.8</td>
<td>82.7</td>
<td>88.0</td>
<td>96.6</td>
<td></td>
</tr>
<tr>
<td>Rh. trifolii 206</td>
<td>49.0</td>
<td>59.0</td>
<td>74.3</td>
<td>91.0</td>
<td>103.1</td>
<td>114.0</td>
<td>121.7</td>
<td></td>
</tr>
<tr>
<td>Rh. trifolii 208</td>
<td>46.0</td>
<td>52.3</td>
<td>57.9</td>
<td>61.9</td>
<td>65.7</td>
<td>69.0</td>
<td>73.6</td>
<td></td>
</tr>
<tr>
<td>Rh. leguminosarum 301</td>
<td>42.7</td>
<td>43.5</td>
<td>43.2</td>
<td>43.2</td>
<td>43.5</td>
<td>43.8</td>
<td>43.6</td>
<td></td>
</tr>
<tr>
<td>Rh. leguminosarum 302</td>
<td>46.5</td>
<td>51.5</td>
<td>57.1</td>
<td>68.7</td>
<td>61.1</td>
<td>59.3</td>
<td>59.0</td>
<td></td>
</tr>
<tr>
<td>Rh. leguminosarum 303</td>
<td>46.2</td>
<td>51.9</td>
<td>61.8</td>
<td>66.4</td>
<td>71.6</td>
<td>74.5</td>
<td>73.8</td>
<td></td>
</tr>
<tr>
<td>Rh. leguminosarum 305</td>
<td>45.5</td>
<td>52.7</td>
<td>59.3</td>
<td>69.4</td>
<td>78.0</td>
<td>90.2</td>
<td>101.6</td>
<td></td>
</tr>
<tr>
<td>Rh. leguminosarum 306</td>
<td>44.5</td>
<td>48.3</td>
<td>51.6</td>
<td>56.5</td>
<td>58.0</td>
<td>62.2</td>
<td>66.1</td>
<td></td>
</tr>
<tr>
<td>Rh. japonicum 410</td>
<td>42.3</td>
<td>43.4</td>
<td>44.1</td>
<td>44.0</td>
<td>43.9</td>
<td>44.2</td>
<td>43.8</td>
<td></td>
</tr>
<tr>
<td>Rh. japonicum 415</td>
<td>42.1</td>
<td>43.1</td>
<td>43.1</td>
<td>43.3</td>
<td>44.7</td>
<td>47.1</td>
<td>50.4</td>
<td></td>
</tr>
<tr>
<td>Rh. japonicum 416</td>
<td>42.3</td>
<td>42.6</td>
<td>42.7</td>
<td>42.6</td>
<td>42.7</td>
<td>42.8</td>
<td>42.8</td>
<td></td>
</tr>
<tr>
<td>Rh. phaseoli 501</td>
<td>45.0</td>
<td>48.5</td>
<td>50.0</td>
<td>51.6</td>
<td>53.9</td>
<td>57.1</td>
<td>65.3</td>
<td></td>
</tr>
<tr>
<td>Rh. phaseoli 503</td>
<td>46.4</td>
<td>50.1</td>
<td>53.2</td>
<td>56.4</td>
<td>60.0</td>
<td>61.8</td>
<td>61.7</td>
<td></td>
</tr>
<tr>
<td>Cowpea organism 602</td>
<td>42.2</td>
<td>43.0</td>
<td>42.6</td>
<td>42.7</td>
<td>42.7</td>
<td>42.7</td>
<td>42.7</td>
<td></td>
</tr>
<tr>
<td>Cowpea organism 603</td>
<td>42.7</td>
<td>43.6</td>
<td>45.0</td>
<td>46.9</td>
<td>47.8</td>
<td>48.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cowpea organism 604</td>
<td>42.7</td>
<td>43.1</td>
<td>42.9</td>
<td>43.3</td>
<td>46.7</td>
<td>47.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dalea organism 701</td>
<td>45.1</td>
<td>46.4</td>
<td>46.9</td>
<td>47.8</td>
<td>47.8</td>
<td>49.1</td>
<td>48.6</td>
<td></td>
</tr>
<tr>
<td>Rh. lupini 801</td>
<td>42.5</td>
<td>43.5</td>
<td>43.2</td>
<td>43.6</td>
<td>43.9</td>
<td>44.3</td>
<td>45.0</td>
<td></td>
</tr>
<tr>
<td>Rh. lupini 802</td>
<td>42.4</td>
<td>43.0</td>
<td>43.8</td>
<td>44.3</td>
<td>45.0</td>
<td>45.6</td>
<td>45.3</td>
<td></td>
</tr>
<tr>
<td>Rh. lupini 803</td>
<td>42.6</td>
<td>43.3</td>
<td>43.5</td>
<td>43.7</td>
<td>43.8</td>
<td>43.6</td>
<td>43.5</td>
<td></td>
</tr>
</tbody>
</table>

In the second test, cultures 302, 303, 503 and 701 apparently reached a maximum viscosity in about 5 days, and no further increases occurred.

The remaining high viscosity cultures showed a steady increase in viscosity up to about 5 days. After this time increases occurred but at a slower rate. Most of the low viscosity cultures remained consistently low, but culture 415 showed a sudden increase during the last 2 days of the period.

EFFECT OF AN EXTENDED INCUBATION PERIOD ON THE VISCOSITY OF SOLUTION CULTURES OF RHIZOBIUM

In view of the results previously secured it became apparent that a longer incubation period might have an effect on the viscosity developed in Rhizobium cultures. An experiment was, therefore, carried out to compare the viscosity of Rhizobium cultures after incubation for 7 and 14 days.

Solution cultures were prepared in the same manner as in the previous experiment. The organisms used are listed in table IX. Five cultures of each of the organisms were prepared by inoculating each of five flasks of sterile medium with 1 cc. of a 72-hour solution culture. After incubating for 7 days at 28 de-
degrees C., three of the cultures were taken for viscosity determinations. The two remaining cultures were incubated an additional 7 days after which the viscosities of the cultures were determined.

The results of this experiment appear in table IX. The data show that with two of the cultures, 410 and 501, there was a higher viscosity after 14 days than was found after 7 days incubation. In culture 501, a rather marked increase in viscosity appeared between the seventh and fourteenth days. Two other cultures, 305 and 205, showed practically the same viscosity at both determinations. The four remaining cultures showed a surprising decrease in viscosity between the seventh and fourteenth days. All of these decreases were of sufficient magnitude to be significant.

**TABLE IX. EFFECT OF THE LENGTH OF THE INCUBATION PERIOD ON THE VISCOSITY OF SOLUTION CULTURES OF RHIZOBIUM.**

<table>
<thead>
<tr>
<th>Culture No.</th>
<th>Length of incubation in days</th>
<th>Average time of flow in seconds</th>
<th>Increase in time of flow in seconds over control</th>
<th>Ratio to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>42.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rh. trifolii</em> 205</td>
<td>7</td>
<td>61.4</td>
<td>18.8</td>
<td>1.44</td>
</tr>
<tr>
<td><em>Rh. trifolii</em> 205</td>
<td>14</td>
<td>61.4</td>
<td>18.8</td>
<td>1.44</td>
</tr>
<tr>
<td><em>Rh. trifolii</em> 206</td>
<td>7</td>
<td>79.2</td>
<td>36.6</td>
<td>1.88</td>
</tr>
<tr>
<td><em>Rh. trifolii</em> 206</td>
<td>14</td>
<td>68.5</td>
<td>25.9</td>
<td>1.68</td>
</tr>
<tr>
<td><em>Rh. leguminosarum</em> 302</td>
<td>7</td>
<td>59.7</td>
<td>17.1</td>
<td>1.40</td>
</tr>
<tr>
<td><em>Rh. leguminosarum</em> 302</td>
<td>14</td>
<td>53.8</td>
<td>11.2</td>
<td>1.26</td>
</tr>
<tr>
<td><em>Rh. leguminosarum</em> 305</td>
<td>7</td>
<td>61.8</td>
<td>19.2</td>
<td>1.45</td>
</tr>
<tr>
<td><em>Rh. leguminosarum</em> 305</td>
<td>14</td>
<td>61.9</td>
<td>19.1</td>
<td>1.45</td>
</tr>
<tr>
<td><em>Rh. japonicum</em> 410</td>
<td>7</td>
<td>44.1</td>
<td>1.5</td>
<td>1.03</td>
</tr>
<tr>
<td><em>Rh. japonicum</em> 410</td>
<td>14</td>
<td>46.5</td>
<td>3.9</td>
<td>1.09</td>
</tr>
<tr>
<td><em>Rh. japonicum</em> 416</td>
<td>7</td>
<td>43.2</td>
<td>0.6</td>
<td>1.01</td>
</tr>
<tr>
<td><em>Rh. japonicum</em> 416</td>
<td>14</td>
<td>42.6</td>
<td>0.0</td>
<td>1.00</td>
</tr>
<tr>
<td><em>Rh. phaseoli</em> 501</td>
<td>7</td>
<td>61.5</td>
<td>18.9</td>
<td>1.44</td>
</tr>
<tr>
<td><em>Rh. phaseoli</em> 501</td>
<td>14</td>
<td>71.8</td>
<td>29.2</td>
<td>1.68</td>
</tr>
<tr>
<td><em>Rh. phaseoli</em> 503</td>
<td>7</td>
<td>53.9</td>
<td>11.3</td>
<td>1.27</td>
</tr>
<tr>
<td><em>Rh. phaseoli</em> 503</td>
<td>14</td>
<td>50.7</td>
<td>8.1</td>
<td>1.19</td>
</tr>
</tbody>
</table>

The test indicates that under the conditions of the experiment a 7-day incubation period is likely to be conducive to the development of a higher relative viscosity than a longer incubation period. Furthermore, the low viscosity cultures do not produce a significant increase in viscosity during the longer incubation period. The 7-day incubation period was, therefore, used in the remaining experiments.

**THE INFLUENCE OF TEMPERATURE ON GUM PRODUCTION**

In connection with a study of the effect of the length of incubation time on viscosity, it became apparent that a study of the
effect of certain environmental factors was desirable. Since the temperature of incubation influences most bacterial metabolic processes it was assumed that temperature might greatly influence the amount of gum produced in Rhizobium cultures.

A temperature of 28 degrees C. was considered as optimum for the growth of the organism. This is in keeping with the findings of some other investigators. Burrill and Hansen (10) concluded that the temperature, optimum for the growth of Rhizobium, lies between 25 and 28 degrees C. Müller and Stapp (41), on the other hand, present experimental evidence to show that the optimum temperature lies between 28 and 30 degrees C.

An experiment was planned in which solution cultures of Rhizobium were grown at temperatures which were below optimum, optimum and above the optimum range. The relative amount of gum produced in these various cultures was then estimated by means of viscosity determinations.

Organisms 206, 305 and 501, which produce a characteristically high viscosity, were chosen for the experiment. The cultures were prepared by placing 100 cc. portions of a glucose-yeast-extract medium into 250 cc. Erlenmeyer flasks. After sterilization, the solutions were inoculated with 1 cc. of a 72-hour culture of the organisms to be tested. Nine cultures of each organism were prepared. Three of the cultures were incubated at 8 degrees C., 3 at 28 degrees and 3 at 32 degrees. After 7 days incubation the viscosities of the cultures were determined.

The results of the test appear in table X. The data show that the incubation temperature has a marked influence on the amount of gum synthesized by Rhizobium. The cultures grown at the optimum temperature, 28 degrees C., produced much more gum than the cultures grown at 32 degrees C. In the cultures incubated at 8 degrees C., growth of the organisms was

<table>
<thead>
<tr>
<th>Culture No.</th>
<th>Temperature in degrees C.</th>
<th>Average time of flow (seconds)</th>
<th>Increase in time of flow over control</th>
<th>Ratio to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rh. trifolii</em> 206</td>
<td>32</td>
<td>42.6</td>
<td>6.0</td>
<td>1.00</td>
</tr>
<tr>
<td><em>Rh. trifolii</em> 206</td>
<td>28</td>
<td>46.6</td>
<td>20.9</td>
<td>1.49</td>
</tr>
<tr>
<td><em>Rh. trifolii</em> 206</td>
<td>8</td>
<td>44.8</td>
<td>2.2</td>
<td>1.05</td>
</tr>
<tr>
<td><em>Rh. leguminosarum</em> 305</td>
<td>32</td>
<td>50.8</td>
<td>8.2</td>
<td>1.19</td>
</tr>
<tr>
<td><em>Rh. leguminosarum</em> 305</td>
<td>28</td>
<td>59.7</td>
<td>17.1</td>
<td>1.40</td>
</tr>
<tr>
<td><em>Rh. leguminosarum</em> 305</td>
<td>8</td>
<td>44.1</td>
<td>1.5</td>
<td>1.04</td>
</tr>
<tr>
<td><em>Rh. japonicum</em> 501</td>
<td>32</td>
<td>46.8</td>
<td>3.7</td>
<td>1.09</td>
</tr>
<tr>
<td><em>Rh. japonicum</em> 501</td>
<td>28</td>
<td>49.3</td>
<td>7.3</td>
<td>1.17</td>
</tr>
<tr>
<td><em>Rh. japonicum</em> 501</td>
<td>8</td>
<td>42.8</td>
<td>0.2</td>
<td>1.01</td>
</tr>
</tbody>
</table>
almost completely inhibited, as shown by the lack of viscosity and the meager development of turbidity.

From these results it seems probable that gum production is a normal process in the metabolism of the organism since it is greatest at the temperature most nearly optimum for the growth of the organism.

THE INFLUENCE OF VARIATIONS IN THE OXYGEN SUPPLY ON GUM PRODUCTION BY RHIZOBIUM

In the previous experiment it was noted that the amount of gum produced was much lower in a culture prepared by placing 100 cc. of medium in a 250 cc. Erlenmeyer flask than in one prepared by placing a similar amount of medium in a 500 cc. Erlenmeyer flask, which indicated an effect of the oxygen supply. In this connection it may be noted that Alicante (1) found that oxygen was a limiting factor in the growth of nodule bacteria in solution, and Allyn and Baldwin (2) reported that the organisms grew only on the surface of strongly reducing media but below the surface in oxidizing media.

A series of experiments were carried out, therefore, to test the influence of the oxygen supply. In the first test, two series of duplicate cultures were prepared. In one series, 100 cc. portions of a glucose-yeast-extract medium were placed into 500 cc. Erlenmeyer flasks. In the second series of cultures, 100 cc. portions of the medium were placed into 250 cc. Erlenmeyer flasks. Following sterilization, the cultures were inoculated with 1 cc. of a 72-hour culture and placed in a 28-degree incubator. After 7 days incubation the viscosities of the cultures were determined.

In the second test, the general procedure was the same as in the first except that a larger number of cultures were employed.

In the third test, in addition to preparing cultures in 500 and 250 cc. Erlenmeyer flasks, a third series of cultures were prepared by placing 100 cc. portions of medium into 125 cc. Erlenmeyer flasks.

| TABLE XI. EFFECT OF SURFACE EXPOSED ON GUM PRODUCTION BY RHIZOBIUM AS MEASURED BY VISCOSITY OF SOLUTION CULTURES. TEST 1. |
|---|---|---|---|
| Culture No. | Size of flask (cc.) | Average time of flow (seconds) | Increase in time of flow over control | Ratio to control |
| Control | 500 | 42.6 | 42.9 | 1.00 |
| *Rh. trifolii* 206 | 500 | 85.5 | 20.9 | 2.01 |
| *Rh. trifolii* 206 | 250 | 63.6 | 1.50 |
| *Rh. leguminosarum* 305 | 500 | 65.8 | 32.2 | 1.54 |
| *Rh. leguminosarum* 305 | 250 | 59.7 | 17.1 | 1.40 |
| *Rh. phaseoli* 501 | 500 | 52.7 | 10.1 | 1.24 |
| *Rh. phaseoli* 501 | 250 | 49.9 | 7.3 | 1.17 |
The results appear in tables XI, XII and XIII. The data in the tables show that the depth of the culture medium and the amount of surface exposed to the air has a very marked influence on the viscosity and, therefore, on the amount of gum produced in solution cultures of Rhizobium. With all of the cultures tested, gum production was much greater when the medium was placed in 500 cc. flasks than in the smaller flasks having a deeper layer of medium. The lack of viscosity, and also turbidity, was especially noticeable in the cultures contained in the 125 cc. flasks.

From these experiments it is apparent that when the legume root-nodule bacteria are placed in a highly reduced medium

### Table XII. Effect of Surface Exposed on Gum Production by Rhizobium as Measured by Viscosity of Solution Cultures. Test 2.

<table>
<thead>
<tr>
<th>Culture No.</th>
<th>Size of flask (cc.)</th>
<th>Average time of flow (seconds)</th>
<th>Increase in time of flow over control</th>
<th>Ratio to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rh. trifolii 205</td>
<td>500</td>
<td>42.6</td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>Rh. trifolii 205</td>
<td>250</td>
<td>61.4</td>
<td>18.8</td>
<td>1.44</td>
</tr>
<tr>
<td>Rh. trifolii 206</td>
<td>500</td>
<td>79.2</td>
<td>36.6</td>
<td>1.86</td>
</tr>
<tr>
<td>Rh. trifolii 206</td>
<td>250</td>
<td>58.6</td>
<td>16.0</td>
<td>1.40</td>
</tr>
<tr>
<td>Rh. leguminosarum 302</td>
<td>500</td>
<td>59.7</td>
<td>17.1</td>
<td>1.40</td>
</tr>
<tr>
<td>Rh. leguminosarum 302</td>
<td>250</td>
<td>55.7</td>
<td>14.1</td>
<td>1.31</td>
</tr>
<tr>
<td>Rh. leguminosarum 305</td>
<td>500</td>
<td>61.8</td>
<td>19.2</td>
<td>1.45</td>
</tr>
<tr>
<td>Rh. leguminosarum 305</td>
<td>250</td>
<td>56.9</td>
<td>14.3</td>
<td>1.34</td>
</tr>
<tr>
<td>Rh. phaseoli 501</td>
<td>500</td>
<td>61.5</td>
<td>18.9</td>
<td>1.44</td>
</tr>
<tr>
<td>Rh. phaseoli 501</td>
<td>250</td>
<td>52.0</td>
<td>9.4</td>
<td>1.22</td>
</tr>
<tr>
<td>Rh. phaseoli 503</td>
<td>500</td>
<td>53.9</td>
<td>11.3</td>
<td>1.27</td>
</tr>
<tr>
<td>Rh. phaseoli 503</td>
<td>250</td>
<td>51.6</td>
<td>9.0</td>
<td>1.21</td>
</tr>
</tbody>
</table>

### Table XIII. Effect of Surface Exposed on Gum Production by Rhizobium as Measured by Viscosity of Solution Cultures. Test 3.

<table>
<thead>
<tr>
<th>Culture No.</th>
<th>Size of flask (cc.)</th>
<th>Average time of flow (seconds)</th>
<th>Increase in time of flow over control</th>
<th>Ratio to control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rh. trifolii 206</td>
<td>500</td>
<td>42.6</td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>Rh. trifolii 206</td>
<td>250</td>
<td>61.7</td>
<td>19.1</td>
<td>1.48</td>
</tr>
<tr>
<td>Rh. trifolii 206</td>
<td>125</td>
<td>51.8</td>
<td>9.2</td>
<td>1.24</td>
</tr>
<tr>
<td>Rh. leguminosarum 305</td>
<td>500</td>
<td>61.0</td>
<td>18.4</td>
<td>1.43</td>
</tr>
<tr>
<td>Rh. leguminosarum 305</td>
<td>250</td>
<td>58.3</td>
<td>15.7</td>
<td>1.37</td>
</tr>
<tr>
<td>Rh. leguminosarum 305</td>
<td>125</td>
<td>49.5</td>
<td>6.9</td>
<td>1.16</td>
</tr>
<tr>
<td>Rh. phaseoli 501</td>
<td>500</td>
<td>52.4</td>
<td>9.8</td>
<td>1.22</td>
</tr>
<tr>
<td>Rh. phaseoli 501</td>
<td>250</td>
<td>48.5</td>
<td>6.9</td>
<td>1.16</td>
</tr>
<tr>
<td>Rh. phaseoli 501</td>
<td>125</td>
<td>48.4</td>
<td>5.8</td>
<td>1.13</td>
</tr>
</tbody>
</table>
such as the glucose-yeast-extract medium used, an abundant supply of oxygen is necessary for gum production and growth. This indicates that gum is probably produced as the result of an oxidative process. The test also gives added weight to the idea that gum production is a normal process in the metabolism of the organism.

**VISCOSITY PRODUCTION IN SOLUTION CULTURES OF RHIZOBIUM AS A SPECIES CHARACTERISTIC**

It was noted in the viscosity tests that some cultures of Rhizobium became relatively viscid after a short period of incubation, while in other cultures the viscosity was only slightly greater than that of the sterile culture medium. The various organisms were found to give consistently high or consistently low viscosities. It appeared that the production of high viscosity might be characteristic of certain species of Rhizobium while the lack of viscosity-producing ability might be characteristic of other species. It seemed, therefore, that the test might have some value as a means of differentiating the various species of Rhizobium and might also throw some light on the genetic relationship between species. With this in mind an extensive study was made of the viscosity production by 59 cultures representing 8 different species of Rhizobium.

In the course of the experiment three different tests were carried out. The procedure used was the same with all of the tests, the only variation being in the strains of Rhizobium studied. One hundred cc. portions of a yeast-extract-glucose solution were placed into 500 cc. Erlenmeyer flasks and subsequently inoculated with 1 cc. of a 72-hour culture of the organisms to be tested. After 7 days incubation at 28 degrees C, the viscosity of each culture was determined. The viscosity readings were made at 28 degrees C.

In the first experiment a limited number of strains of Rhizobium was studied. In the second experiment the viscosity-producing power of the remainder of the laboratory collection of Rhizobium cultures was determined. In the third experiment the viscosity-producing power was determined for all of the cultures in the laboratory collection, with the exception of 129, 202 and 306 which were inadvertently omitted. The third test was made in order to verify the results secured in the two previous tests.

In order to summarize the results of the three experiments, the times of flow in seconds and ratio values for the three tests have been incorporated in table XIV.

An examination of the data in this table shows, at least with the cultures used, that the legume nodule bacteria can be divided into two groups on the basis of the viscosity produced in glucose-yeast-extract solution cultures.
The first group includes the cultures of *Rh. meliloti*, *Rh. japonicum*, *Rh. lupini* and the cowpea bacteria. These organisms produced little or no increase in viscosity over that of the sterile medium. In general, taking the relative viscosity of the sterile medium as 1.00, the viscosity of the cultures of this group was below 1.10.

| TABLE XIV. COMPARATIVE VISCOSITIES OF SOLUTION CULTURES OF SPECIES AND STRAINS OF RHIZOBIUM. SUMMARY OF THE RESULTS OF TESTS 1, 2 AND 3. |
|---|---|---|---|---|
| Culture No. | Test 1 | Test 2 | Test 3 |
| | Average time of flow (seconds) | Ratio to control | Average time of flow (seconds) | Ratio to control | Average time of flow (seconds) | Ratio to control |
| **Control** | 42.6 | 1.00 | | | | |
| *Rhizobium meliloti* cultures |
| 101 | 45.4 | 1.06 | 44.7 | 1.05 |
| 103 | 48.3 | 1.02 | 43.3 | 1.02 |
| 105 | 44.0 | 1.03 | 44.4 | 1.04 |
| 107 | 43.7 | 1.02 | 43.8 | 1.02 |
| 108 | 43.6 | 1.02 | 43.6 | 1.02 |
| 110 | 43.5 | 1.02 | 44.0 | 1.03 |
| 114 | 44.6 | 1.05 | 43.7 | 1.02 |
| 115 | 43.0 | 1.01 | 45.2 | 1.06 |
| 118 | 43.7 | 1.02 | 43.5 | 1.02 |
| 119 | 44.5 | 1.04 | 45.2 | 1.06 |
| 124 | 46.3 | 1.09 | 45.0 | 1.06 |
| 121 | 45.4 | 1.06 | 44.0 | 1.03 |
| 127 | 43.2 | 1.01 | 44.9 | 1.05 |
| 128 | 43.5 | 1.02 | 43.9 | 1.03 |
| 129 | 43.8 | 1.03 | 43.9 | 1.03 |
| 130 | 44.8 | 1.05 | | | |
| 131 | 45.8 | 1.07 | | | |
| 132 | 44.5 | 1.04 | | | |
| 133 | 43.9 | 1.03 | | | |
| *Rhizobium trifolii* cultures |
| 201 | 57.3 | 1.34 | 55.0 | 1.29 |
| 202 | 53.1 | 1.25 | | |
| 203 | 55.8 | 1.31 | | 51.2 | 1.20 |
| 205 | 78.8 | 1.85 | | 75.0 | 1.76 |
| 206 | 85.1 | 2.00 | 104.4 | 2.45 | 90.4 | 2.11 |
| 208 | 68.3 | 1.60 | | | 55.0 | 1.29 |
| *Rhizobium leguminosarum* cultures |
| 301 | 44.1 | 1.03 | | | 44.0 | 1.03 |
| 302 | 58.2 | 1.36 | | | 54.2 | 1.27 |
| 303 | 62.7 | 1.47 | | | 56.0 | 1.31 |
| 305 | 75.2 | 1.76 | | | 77.0 | 1.81 |
| 306 | 65.6 | 1.49 | | | | |
TABLE XIV—(Continued)

<table>
<thead>
<tr>
<th>Culture No.</th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average time of flow (seconds)</td>
<td>Ratio to control</td>
<td>Average time of flow (seconds)</td>
</tr>
<tr>
<td>Rhizobium japonicum cultures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>401</td>
<td>......</td>
<td>......</td>
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<td>......</td>
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<td>44.3</td>
</tr>
<tr>
<td>410</td>
<td>43.2</td>
<td>1.01</td>
<td>......</td>
</tr>
<tr>
<td>411</td>
<td>......</td>
<td>......</td>
<td>44.3</td>
</tr>
<tr>
<td>412</td>
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<td>416</td>
<td>42.7</td>
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<td>42.7</td>
</tr>
<tr>
<td>Rhizobium phaseoli cultures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>501</td>
<td>56.0</td>
<td>1.31</td>
<td>54.5</td>
</tr>
<tr>
<td>503</td>
<td>55.8</td>
<td>1.31</td>
<td>49.7</td>
</tr>
<tr>
<td>504</td>
<td>......</td>
<td>......</td>
<td>......</td>
</tr>
<tr>
<td>Cowpea cultures</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>603</td>
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<td>1.06</td>
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<td>604</td>
<td>48.2</td>
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<tr>
<td>609</td>
<td>......</td>
<td>......</td>
<td>42.7</td>
</tr>
<tr>
<td>Dalea culture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>701</td>
<td>48.2</td>
<td>1.13</td>
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</tr>
<tr>
<td>Rhizobium lupini cultures</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>801</td>
<td>43.3</td>
<td>1.02</td>
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</tr>
<tr>
<td>802</td>
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<td>1.02</td>
<td>......</td>
</tr>
<tr>
<td>803</td>
<td>43.2</td>
<td>1.01</td>
<td>......</td>
</tr>
</tbody>
</table>

The second group includes cultures of *Rh. trifolii*, *Rh. leguminosarum*, *Rh. phaseoli* and the Dalea bacteria. These organisms produced a relatively high degree of viscosity compared with that of the sterile medium. The ratios of the viscosity of the cultures compared with that of the medium ranged from slight-
ly more than 1.10 to nearly 2.50. Most of the values were between 1.20 and 1.80.

The above grouping holds very definitely for 114 of the 118 values given in the table. Aberrant results were secured with culture 301 in two tests, and with cultures 604 and 415 in one test. Cultures 604 and 415 were evidently contaminated. Culture 301 was apparently atypical as shown by the character of its growth in milk.

Not only can the various species be grouped on the basis of their viscosity producing power, but the viscosity producing power of the individual strains within a species also appears to be relatively constant. An analysis of the data shows that in 50 of the 59 cultures the ratios secured in separate tests agreed very closely. This constancy of viscosity becomes even more striking when the fact is considered that nearly 2 months elapsed between the first and third tests.

The differences observed in the viscosity producing power of various species may be due to actual differences in the amount of gum produced by a given species of Rhizobium or to differences in the chemical nature of the gum.

A recent study by Heidelberger and Kendall (31) on Pneumococcus gum indicates a relationship between viscosity production and the frequency of occurrence of the carboxyl group in the polysaccharide chain. Furthermore, it has been shown by Hopkins, Peterson and Fred (34) that there are differences in the uronic acid content of gums produced by different species of Rhizobium which apparently means differences in the frequency of occurrence of carboxyl groups in the gum complex.

In the tests reported here the viscosity of the cultures of *Rh. trifolii* and *Rh. leguminosarum* was much greater than that of cultures of *Rh. meliloti*; and since the latter showed a much smaller content of uronic acid than the other species according to Hopkins, Peterson and Fred—and therefore a smaller number of carboxyl groups—it appears that the conclusion of Heidelberger and Kendall may be applicable to Rhizobium and that differences in the chemical nature of the gum may have more influence on the viscosity of solution cultures of Rhizobium than do differences in amount of gum produced.

**RHIZOBIUM GUM AS A SOURCE OF ENERGY FOR THE ORGANISM**

It has been shown that gum may be a normal product of the metabolic activities of Rhizobium and may also serve as a source of reserve food for the organism. The object of this experiment was to determine whether or not Rhizobium is able to utilize the purified gum as an energy source.

Gum was isolated from a 28-day old culture of organism 302
in mannitol-yeast-extract medium by precipitation with alcohol and separation by filtration. The gum thus secured was then purified by dissolving and reprecipitating three times. The purified gum, when dry, formed a brittle gray-white solid.

As a basic medium a series of yeast-extract agar slants were prepared. The gum was powdered, suspended in water and sterilized. A sufficient amount of this suspension was then added to the tubes containing the yeast-extract agar to provide a concentration of 0.5 percent. To a second series of tubes, sufficient glucose was added to give a concentration of 0.5 percent. A third series of tubes, containing the yeast-extract agar without added carbohydrate was used as a control.

The media thus prepared were divided into two groups, each containing two tubes of gum agar, two tubes of glucose agar and two tubes of carbohydrate-free agar. One group of tubes was then streaked with growth from an agar slant of culture 302 and the second group was streaked with growth from culture 416.

The cultures were then incubated at 28 degrees C. Observations of the amount of growth were made daily for 8 days. Growth was obtained on all of the inoculated tubes, including the agar control. Both organisms, however, grew vigorously on the glucose agar while very little growth occurred on the other two media. Growth developed at about the same rate on both the gum agar and the carbohydrate-free agar. A description of the nature of growth secured after 8 days incubation is tabulated below:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Medium</th>
<th>Description of growth</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Rh. leguminosarum</em> 302</td>
<td>Glucose agar</td>
<td>Extremely heavy growth covering entire slant</td>
</tr>
<tr>
<td><em>Rh. leguminosarum</em> 302</td>
<td>Gum agar</td>
<td>Thin line of growth along streak</td>
</tr>
<tr>
<td><em>Rh. leguminosarum</em> 302</td>
<td>Agar containing no added carbohydrate</td>
<td>Thin line of growth along streak</td>
</tr>
<tr>
<td><em>Rh. japonicum</em> 416</td>
<td>Glucose agar</td>
<td>Fairly heavy growth along streak covering about half the width of the slant</td>
</tr>
<tr>
<td><em>Rh. japonicum</em> 416</td>
<td>Gum agar</td>
<td>Faint line of growth along streak</td>
</tr>
<tr>
<td><em>Rh. japonicum</em> 416</td>
<td>Agar containing no added carbohydrate</td>
<td>Faint line of growth along streak</td>
</tr>
</tbody>
</table>
From the results it is evident that under the conditions of the experiment the gum was not utilized by the organisms. It is probable, however, that the properties of the gum might have been changed during the process of isolation making it unsuitable as an energy source for the organisms. This was indicated by the fact that the dry gum was almost insoluble. On the other hand, if the properties of the gum were unchanged during the process of isolation the results may be taken as an indication that gum is not a reserve food material but rather a by-product of fermentation which is not utilizable.

SUMMARY AND CONCLUSIONS

1. The purposes of the investigations described in this bulletin were: (a) to develop a rapid method for measuring gum production by Rhizobium, (b) to determine the influence of environmental factors on gum production, (c) to study the rate of gum production, (d) to compare the amounts of gum produced by different species of Rhizobium, and (e) to study the role of gum production in the metabolism of the organism.

2. In preliminary tests the gravimetric method used by some investigators for determining the gum content of bacterial cultures was found to be very time-consuming. A more rapid method, which would yield at least relative results was therefore sought. Measurements of the viscosity of Rhizobium cultures showed that certain organisms produced a marked increase in the viscosity of their solution cultures while other cultures failed to produce any appreciable increases. With the high viscosity cultures the viscosity developed was found to be directly proportional to the amount of gum present in the solution. Viscosity measurements were therefore used to determine the relative amount of gum present in solution cultures of these high viscosity-producing organisms.

3. A comparison was made of the viscosity of solution cultures of Rhizobium previous to and following vigorous shaking. In the cultures tested the viscosity was found to be entirely unchanged by shaking, thus indicating that the gum does not form a structure such as that present in a gelatine solution and that the gum is probably an integral part of the solution rather than existing as a capsule around the bacterial cell.

4. Additions of small amounts of acid or alkali to the solution cultures failed to produce any measurable changes in the viscosity of the cultures.

5. Regardless of whether the cultures received a light or heavy inoculation their viscosities were found to be the same at
the end of a 7-day incubation period. This was taken to indicate that for a given concentration of sugar, an equilibrium may exist between the amount of gum produced, the amount of sugar transformed and probably the number of organisms developing. It was thought that this point was reached in all of the cultures during the 7-day incubation period.

6. A study of daily increases in the gum content of certain cultures of Rhizobium showed that the accumulation of gum proceeded steadily in all of the cultures up to the fifth day. At this point some of the cultures showed no further increase in gum content, while the others showed a decrease in the rate at which gum was being formed.

7. A comparison was made of the gum content of solution cultures of Rhizobium after 7 days incubation and after 14 days incubation. Some of the cultures tested showed approximately the same gum content at both 7 and 14 days while others showed a decrease in gum content between the seventh and fourteenth days.

8. In a study of the effect of temperature on gum production, it was found that considerably more gum was produced at the optimum temperature than at temperatures above or below the optimum. This was taken as an indication that gum production is probably a normal process in the metabolism of the organism.

9. In order to determine the relationship of oxygen supply to gum production, cultures of Rhizobium were grown in 500 cc., 250 cc. and 125 cc. Erlenmeyer flasks, each containing 100 cc. of medium. In all of the cultures tested the amount of gum produced was found to be largest in the flasks allowing the largest surface exposure, and the amount produced became increasingly smaller as the amount of surface area exposed was decreased. Since the organisms are rather strongly aerobic the results lend added weight to the assumption that the gum is produced as a normal product of metabolism.

10. Viscosity tests showed that certain cultures of Rhizobium produced marked increases in the viscosity of their solution cultures, while with other cultures very little increase in viscosity was observed. Since the ability to produce a viscid solution seemed to be characteristic of certain species of Rhizobium, it was thought that differences in viscosity-producing power might have some value in differentiating the Rhizobium species. Viscosity tests were made, therefore, on 59 strains of Rhizobium representing 8 different species. The cultures fell into two groups on the basis of their ability to increase the viscosity of a glucose-yeast-extract medium. Organisms of the
first group produced little or no increase in the viscosity of the culture solution. The group included strains of *Rh. meliloti*, *Rh. japonicum*, *Rh. lupini* and the cowpea bacteria. The organisms of the second group produced a marked increase in the viscosity of the culture solutions. The viscosities of these cultures ranged from 1.2 to 2.4 times that of the sterile medium. The group includes *Rh. trifolii*, *Rh. leguminosarum* and *Rh. phaseoli* and probably the Dalea bacteria. Differences in the chemical nature of the gums produced by the various species of *Rhizobium* were thought to play an important part in determining the ability of these organisms to increase the viscosity of the solution cultures.

11. Purified gum was used as a carbohydrate source for the growth of *Rhizobium*, but under the conditions of the experiment it was not utilized by the organisms.

**LITERATURE CITED**


