AN AEROBIC SOIL MICROORGANISM WHICH DECOMPOSES
BLOOD GROUP SUBSTANCES

I. Metabolic and Immunochemical Studies

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A number of microorganisms have the ability
to degrade cellulose, agar, bacterial polysaccha-
drides, and blood group substances. The
Myxococcus of Morgan and Thaysen (1933) was
shown to split blood group A substance in horse
saliva but not the A substance in peptone (Land-
steiner and Chase, 1935a, b). It also attacked the
specific polysaccharide of Shigella dysenteriae
(Shiga and Flexner), pneumococcus type II, and
Mycobacterium tuberculosis. Saccharobacterium
ovale, isolated by Sickles and Shaw (1934), split
blood group A substance and type II pneumo-
coccal polysaccharide (Landsteiner and Chase,
1934b). Chase (1938) reported the isolation from
leaf mold of a gram-negative cocccobacillus which
grew well in a basal salt solution (0.1 per cent
(NH₄)₂SO₄, 0.2 per cent K₂HPO₄) containing
blood group A substance, the specific serological
activity of which disappeared as a result of
bacterial multiplication. In this paper are re-
ported the results of further investigation into
the metabolism of this latter organism, with
particular reference to its effect on blood group
substances. Chase’s original observations have
been confirmed, and it has been shown that
growth in solutions of blood group substance
results not only in the disappearance of serologic
activity but also in the decomposition of the
substances, with utilization of their individual sugar
components by the organism.

MATERIALS AND METHODS

Preparation of purified substrates. Blood group
A and O(H) substances were obtained from the
mucosal linings of individual hog stomachs by

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versity.

peptic digestion, phenol extraction, and precipi-
tation from phenol solution with ethanol at a
concentration of 10 per cent (v/v) (Morgan and
King, 1943; Bendich et al., 1946). Of 19 prepara-
tions, 11 showed only O(H), 4 showed only A,
and 3 showed both A and O(H) activity. Mucin
blood group substance (A + O(H)) was prepared
from hog gastric mucin (Wilson) by the same
procedure. Horse blood group B substance was
isolated from individual horse stomach linings by
the procedure of Baer et al. (1950). Of 6 horse
stomach digests, 1 showed only A activity, 3
both A and B activity, 1 neither A nor B, and
1 (horse G4) only B activity. Water soluble
human blood group B substance was prepared from
the sputum of a B secretor by peptic and
trypctic digestion followed by phenol extraction,
the most potent fraction being insoluble in
phenol. A sample of type XIV pneumococcal
capsular polysaccharide, isolated and purified by
a combination of the procedures of Heidelberger
et al. (1936) and Brown (1939) (see Kabat and
Mayer, 1948), was kindly supplied by Dr. E. A.
Kabat.

Mucin blood group substance medium was
prepared by dissolving purified mucin blood
group substance in basal salt solution (0.1 per
cent (NH₄)₂SO₄ and 0.2 per cent K₂HPO₄) to give
a final concentration of 0.1 per cent at pH 7.2.
Mucin blood group substance was also incor-
porated in solid medium (2 per cent agar) in a
concentration of 1 per cent at pH 7.2.

Serological and immunochemical methods. Anti-
sera to purified blood group substances were
prepared by the injection of human volunteers
(Kabat and Bezer, 1945). Serum Mer and serum
Mil were obtained from donors of type O after
the subcutaneous injection of 1 mg of blood
group A substance from a single hog stomach
devoid of O(H) activity. Serum Heg was ob-
tained from a type O donor and serum Stu from
a type A donor after the subcutaneous injection of 1 mg of human B substance.

Blood group A and B antigens were estimated by inhibition of hemagglutination and determined quantitatively (Heidelberger and MacPherson, 1943) by precipitation of specific antibody from calibrated antisera (Kabat and Bezer, 1945; Kabat et al., 1946). Blood group O(H) antigen was estimated by inhibition of hemagglutination using group O erythrocytes and bovine serum containing anti-O(H), previously absorbed with A1B erythrocytes (Bendich et al., 1947). Quantitative estimation of O(H) antigen is not possible. Type XIV pneumococcal polysaccharide was estimated by inhibition of agglutination of type A1 erythrocytes by rabbit anti-SXIV. Hexosamine was determined by the method of Elson and Morgan (1933), methylpentose by the method of Dische and Shettles (1948), reducing sugar, referred to glucose, by the method of Hagedorn and Jensen (1923), and nitrogen by the Markham (1942) modification of the micro-Kjeldahl procedure. N-Acetylhexosamine was determined by the Morgan and Elson method as modified by Aminoff et al. (1952).

**EXPERIMENTAL RESULTS**

*Morphology and cultural characteristics.* Organisms were recovered on mucin blood group substance agar from a lyophilized culture received from Dr. Merrill W. Chase of the Rockefeller Institute for Medical Research. A single cell isolate was obtained which in subculture corresponded precisely in all its morphological and cultural characteristics with the original description of Chase (1938). In mucin blood group substance medium the optimum temperature for growth was between 27 and 32°C with complete inhibition at 38°C (figure 1). Growth occurred over a wide pH range, but was maximal within the range 6.2 to 6.9 (figure 2). There was slight acceleration of growth in mucin blood group substance broth with the addition of (NH₄)₂SO₄ up to a concentration of 0.5 per cent, definite inhibition occurring above this concentration.

**Growth in relation to mucin blood group substance concentration.** Solutions of mucin blood group substance in concentrations from 0.01 mg per ml to 20 mg per ml in basal salt solution were inoculated with a washed suspension. After incubation at 30°C for 6 days, growth and pH of the medium were measured, and culture supernatants were tested for residual blood group A activity by hemagglutination inhibition. Blood group A activity was no longer detected in cultures which at the outset had 2.5 mg mucin blood group substance per ml, or less, and which after 6 days showed measurable turbidity indicative of growth. Figure 3 shows that a direct correlation exists between the amount of blood group substance present and the final amount of growth as measured turbidimetrically. With concentrations of mucin blood group substance greater than 2.5 mg per ml, growth progressed sufficiently to lower the pH of the medium to inhibitory levels (i.e., pH 6.0 or less, figure 2), but then ceased before the destruction of the A activity of mucin blood group substance was complete. To determine whether cessation of growth was in fact due to the accumulation of acid despite the presence of buffer, the pH of the solutions originally containing 20 and 10 mg of mucin blood group substance per ml was readjusted at intervals to 7.0. Growth continued and was accompanied by the complete destruction of blood group activity.

**Substrates utilized for growth.** A number of polysaccharides as well as partially and completely degraded mucin blood group substances

![Figure 1. Effect of temperature on growth in mucin blood group substance medium.](image-url)
were studied for their ability to support growth. The intact polysaccharides were dissolved in the basal salt solution (0.1 per cent \(\text{(NH}_4\text{)}_2\text{SO}_4\), 0.2 per cent \(\text{K}_2\text{HPO}_4\)) to give a final concentration of 0.1 per cent. Hog A (G 15), hog O(H) (G3), horse, and human B substances all supported growth which was accompanied by the disappearance of each group antigen, as determined by hemagglutination inhibition. In addition, serologically inactive cow (cow 22) and horse (horse 6) substances (kindly supplied by Dr. E. A. Kabat) supported growth, whereas pneumococcal SXIV in basal salt solution did not. Partially and completely degraded blood group substances in the same concentration as the intact substances above were incorporated into basal salt solution. Completely hydrolyzed mucin blood group substance (2 n HCl, 2 hr, 100 C) whether subsequently neutralized or dried to remove HCl, failed completely to support growth. Partially hydrolyzed mucin blood group substance, however (pH 1.5, 100 C, 2 hr, 4 hr, neutralized) supported good growth, even though blood group A activity had been destroyed, as did concentrated dialyzates of these same materials. A sample of mucin blood group substance reisolated after digestion with enzymes of Clostridium tertium (Howe et al., 1957) also supported growth, although the A antigen had been inactivated by the prior enzyme treatment.

Combinations of galactose with glucosamine and L-fucose, or galactosamine and L-fucose, in basal salts did not allow growth, whereas N-acetylglucosamine alone or in combination with galactose or galactosamine and fucose in basal salts did. In two experiments using a medium consisting of 0.2 per cent N-acetylglucosamine, 0.1 per cent \(\text{(NH}_4\text{)}_2\text{SO}_4\), and 0.2 per cent \(\text{K}_2\text{HPO}_4\), abundant growth was accompanied in one instance by reduction of N-acetylglucosamine and glucosamine color values each to 5 per cent and

**Figure 2.** Effect of initial pH on growth in mucin blood group substance medium. (Optical density measured in Coleman nephocolorimeter with optical filter 525 m\(\mu\).)

**Figure 3.** Relation of mucin blood group substance (BGS) concentration to growth. (Optical density measured in Coleman nephocolorimeter with optical filter 525 m\(\mu\).)
reducing sugar to 15 per cent of the corresponding control values. In the second experiment, growth was less, and the amino sugar values were reduced to 50 per cent and the total reducing sugar to 32 per cent of the corresponding control values.

**Effects on human erythrocytes of actively growing mucin blood group substance cultures and of bacterial suspensions in saline.** Experiments were designed to detect the action of growing cultures and saline suspensions of bacterial cells on erythrocytes. Sterile washed erythrocytes were suspended in mucin blood group substance medium to a concentration of 1.5 per cent by volume. This mixture was inoculated with 0.1 ml of an 18-hr mucin blood group substance culture and incubated at 30 C. Samples were taken at 24 and 48 hr, and the erythrocytes separated out by low speed centrifugation. After being washed five times with saline to remove traces of soluble blood group substance, they were tested with a variety of antisera. As controls, unoinoculated solutions of mucin blood group substance containing erythrocytes in suspension were treated in an identical fashion. The results are summarized in table 1. Erythrocytes were also exposed in a similar manner to suspensions of washed bacterial cells from 24- and 48-hr mucin blood group substance cultures in order to detect the effect of resting bacterial cells on erythrocyte antigens. The results are summarized in table 2. It will be seen that erythrocytes of all three groups whether exposed to actively growing (table 1) or to resting (table 2) bacterial cells became agglutinable by each corresponding donor's plasma (panagglutinable). Panagglutinin of this kind was also present in the anti-A and anti-B sera, but in a concentration too low to mask the specific isoagglutinin readily demonstrable at the higher dilutions used. The agglutination of erythrocytes in anti-O(H) after exposure either to cultures or to resting cells could have been due either to true anti-O(H) or to panagglutinin, as the titers of each in the bovine serum used were about equal. The M antigen was inactivated only after exposure to actively growing cultures, and not after exposure, even for 48 hr, to resting bacteria. The M antigen survived both treatments.

**Utilization of A, B, O(H), and mucin blood group substances.** Solutions of purified blood group substance dissolved at a concentration of 0.1 per cent in phosphate buffer or basal salt solution were sterilized by autoclaving. The inoculum, per 100 ml of medium, comprised the washed bacterial cells harvested from 1 ml of a fully grown mucin blood group substance broth culture. After incubation at 30 C, the cultures in purified blood group substance were centrifuged, the sediment washed three times in saline, and the washings combined with the supernatants. The supernatants were dialyzed against six daily changes of deionized water and the dialyzates

### TABLE 1

Effect of growing cultures on erythrocytes

<table>
<thead>
<tr>
<th>Donors</th>
<th>Grouping</th>
<th>Period of Incubation with Culture, Hr</th>
<th>Anti-A*</th>
<th>Anti-O(H)†</th>
<th>Anti-B‡</th>
<th>Anti-M</th>
<th>Anti-N</th>
<th>Same donor's plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 37353 and G11</td>
<td>A; MN</td>
<td>24</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td>Control</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No. 36866 and no. 38666</td>
<td>B, MN</td>
<td>24</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No. 37377 and no. 37220</td>
<td>O, MN</td>
<td>24</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<td></td>
<td></td>
<td>Control</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* Serum Mi14 1/40.
† Bovine serum Gi1 1/2.
‡ Serum Stu 1/20.
TABLE 2

Effect of washed bacterial cell suspensions on erythrocytes

<table>
<thead>
<tr>
<th>Donor</th>
<th>Grouping</th>
<th>Age of Bacterial Cells, Hr.</th>
<th>Agglutination with:</th>
<th>Same donor’s plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anti-A*</td>
<td>Anti-O(H)†</td>
</tr>
<tr>
<td>No. 37353</td>
<td>A, N</td>
<td>18</td>
<td>+</td>
<td>−</td>
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<td></td>
<td>Control</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>No. 37377</td>
<td>O, MN</td>
<td>18</td>
<td>+</td>
<td>+</td>
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<td>Control</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>DeBel</td>
<td>B, M</td>
<td>18</td>
<td>−</td>
<td>+</td>
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<tr>
<td></td>
<td></td>
<td>Control</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>−</td>
<td>+</td>
</tr>
</tbody>
</table>

* Serum Milb 1/40.
† Bovine serum G1c.
‡ Serum Stu 1/20.

concentrated under reduced pressure at 48°C and made up to 10 ml. The nondialyzable residues were recovered by lyophilization and dried to constant weight in vacuo over P₂O₅. Dialyzates and nondialyzable residues were analyzed for hexosamine and reducing sugar after hydrolysis (2 x HCl, 2 hr, 100°C) and neutralization. Nondialyzable residues were analyzed for methylpentose, and dialyzates for methylpentose, reducing sugar, and N-acetylated hexosamine, without preliminary hydrolysis. Loss of blood group activity was detected in all experiments by hemagglutination inhibition, and was measured quantitatively by the precipitin technique in those experiments in which A or B substances served as substrate. Samples of uninoculated medium served as controls.

Data from seven experiments are presented in table 3. Growth on each of the substrates was accompanied by a marked reduction in the amount of nondialyzable material recovered, which decreased with continued growth. This is shown particularly in experiment VI in which O(H) substance served as substrate. Three identical cultures incubated for 4, 7, and 18 days, respectively, and a control solution incubated for 7 days, were employed in this experiment. Of the original 54 mg, 35 per cent was recovered as nondialyzable residue after 4 days' growth, 9 per cent after 7 days, and less than 1 per cent after 18 days.

The content of hexosamine, reducing sugar, and methylpentose of the nondialyzable residues recovered from inoculated solutions of each of the blood group substances was much lower than that of the nondialyzable residues of uninoculated control solutions. The latter of course represented unchanged substrate, the analytical properties of which were identical with those of the original blood group substance. With longer periods of incubation, this difference became greater. For example, in experiment V (O(H) substance), after 5 days of growth, the methylpentose content was 2 per cent, the reducing sugar 22 per cent, and the hexosamine 8 per cent, the corresponding control values being 8, 50, and 27 per cent, respectively. The possible contribution to the nondialyzable residue made by the organism during growth must be considered negligible, since in experiment VI, the residue of culture no.
**Table 3**

**Metabolic degradation of blood group A, B, and O(H) substances**

<table>
<thead>
<tr>
<th>Experiment No.; Substrate Preparation; [Antigen (a)].</th>
<th>Substrate*</th>
<th>Days Incubated 30°C</th>
<th>Nondialyzable Residue</th>
<th>Total Recovery of Dialyzable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total recovery</td>
<td>Hexosamine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Of original</td>
<td>%</td>
</tr>
<tr>
<td>I; Hog mucin; [A + O(H)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculated...</td>
<td>100</td>
<td>2</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Uninoculated...</td>
<td>100</td>
<td>2</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>II; Hog G15; [A]</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculated...</td>
<td>60</td>
<td>4</td>
<td>47</td>
<td>78</td>
</tr>
<tr>
<td>Uninoculated...</td>
<td>60</td>
<td>4</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Inoculated...</td>
<td>60</td>
<td>8</td>
<td>22</td>
<td>37</td>
</tr>
<tr>
<td>Uninoculated...</td>
<td>60</td>
<td>8</td>
<td>58</td>
<td>97</td>
</tr>
<tr>
<td>III; Hog G16; [A]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculated...</td>
<td>44</td>
<td>4</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>Uninoculated...</td>
<td>44</td>
<td>4</td>
<td>43</td>
<td>98</td>
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<tr>
<td>IV; Hog G3; [O(H)]</td>
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<tr>
<td>Inoculated no. 1</td>
<td>47</td>
<td>2</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>Inoculated no. 2</td>
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<td>19</td>
</tr>
<tr>
<td>Uninoculated...</td>
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<td>4</td>
<td>47</td>
<td>100</td>
</tr>
<tr>
<td>V; Hog G14; [O(H)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inoculated...</td>
<td>69</td>
<td>5</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Uninoculated...</td>
<td>69</td>
<td>5</td>
<td>68</td>
<td>97</td>
</tr>
<tr>
<td>VI; Hog G10; [O(H)]</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Inoculated no. 1</td>
<td>54</td>
<td>4</td>
<td>19</td>
<td>35</td>
</tr>
<tr>
<td>Inoculated no. 2</td>
<td>54</td>
<td>7</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Inoculated no. 3</td>
<td>54</td>
<td>18</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Uninoculated...</td>
<td>54</td>
<td>7</td>
<td>52</td>
<td>97</td>
</tr>
<tr>
<td>VII; RS₆; [B]</td>
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</tr>
<tr>
<td>Inoculated...</td>
<td>47</td>
<td>5</td>
<td>10</td>
<td>21</td>
</tr>
<tr>
<td>Uninoculated...</td>
<td>47</td>
<td>5</td>
<td>47</td>
<td>100</td>
</tr>
</tbody>
</table>

* (NH₄)₂SO₄ (100 mg) added to all flasks in experiment I, 20 mg in experiment III, and 50 mg in experiment V; none added in experiments II, IV, VI, and VII.

† HCl (2 n) 2 hr, 100 C, neutralized.

3, incubated for 18 days, was only 1 per cent of the original weight of substrate. It follows that in cultures incubated for shorter periods of time contamination with nondialyzable products of bacterial metabolism would have been even less.

The nondialyzable residues recovered after growth were found to retain little or no specific serological activity. In experiment I, with mucin blood group substance as substrate, the nondialyzable residue recovered after 2 day growth
was completely devoid of A activity as evidenced by failure of 560 µg to inhibit hemagglutination, as compared with the control value of 4 µg. In experiment III, in which blood group A substance served as the substrate, 430 µg of the nondialyzable residue recovered after 4 day growth failed to inhibit hemagglutination as compared with a control value of 4 µg. In experiment VII, in which B substance served as substrate 480 µg failed to inhibit hemagglutination as compared with a control value of 4 µg. Other experiments gave similar results, growth being accompanied by a reduction and eventual loss of specific blood group activity of the substrate. As clearly shown in experiment VI, however, the microorganism continued to grow in the presence of serologically inactive blood group substance, which was then further degraded. No blood group activity was found in any of the dialyzates when tested by hemagglutination inhibition. The capacity of the nondialyzable residues in experiments I, II, and III to precipitate anti-A and in experiment VII to precipitate anti-B is shown in figures 4 and 5. For experiment I the curve labeled “hog mucin, no growth 2 days” is to be compared with the curve labeled “hog mucin, growth 2 days.” It will be seen that the latter material had lost all blood group activity since it no longer precipitated anti-A.

In the second experiment, utilizing A substance from a single hog stomach, the control material (hog A, G15, no growth) precipitated more anti-A per microgram since it lacked the O(H) substance present in mucin. Comparison of this curve with the curve labeled “hog A, G15 growth 4 days” shows that the blood group A activity of the nondialyzable material recovered after 4 day growth was markedly reduced. For example, 21 µg of the nondialyzable control residue precipitated 22 µg of antibody N as compared with 10 µg precipitated by the nondialyzable material recovered after 4 day growth. After 8 day growth (hog A, G15 growth 8 days), up to 50 µg of the nondialyzable material failed to precipitate any anti-A. Much greater amounts of antigen (251, 503, and 1070 µg) each gave a negligible amount of precipitate. In experiment III in which (NH₄)₂SO₄ had been added to blood group A substance from a single hog stomach (G16), complete destruction of the A antigen took place during growth within 4 days, as shown by the failure of the nondialyzable residue to precipitate appreciable amounts of anti-A (hog A, G16 growth 4 days).

Figure 5 shows virtually complete destruction of B antigen in the nondialyzable residue recovered after 5 day growth on B substance. Although 19 µg of the control material (B substance, no growth) precipitated 20 µg of antibody N, 5 times as much nondialyzable residue (100 µg) precipitated but 3 µg antibody N.

Analysis of the dialyzates for total hexosamine, reducing sugar, methylpentose, and N-acetylhexosamine showed that each of these components originating from the blood group substance had in large measure been consumed. For example, in experiment III, 44 mg of A substance were used, 30 per cent of which (13 mg) was hexosamine, 55 per cent (24 mg) reducing sugar, and 7 per cent (3 mg) methylpentose (see control values). After 4 day growth, only 10 mg of nondialyzable material were recovered, 20 per cent

![Figure 4.](http://jb.asm.org) The capacity of nondialyzable residues of experiments I (hog mucin), II (single hog G15), and III (single hog G16) to precipitate anti-A (see table 3).
(2 mg) of which represented hexosamine, 40 per cent (4 mg) reducing sugar, and 4 per cent (0.4 mg) methylpentose. The other experiments, regardless of the blood group specificity of the substrate, gave similar results. The deficits of hexosamine, reducing sugar, and methylpentose could not be accounted for in the test dialyzates since only small amounts of each component were detected. It seems logical to deduce therefore that these constituents of blood group substances had been utilized during growth. The presence of trace amounts of sugars in uninoculated control dialyzates probably resulted from slight degradation during autoclaving.

Absence of enzymes in filtrates and culture supernatant fluids. Cultures in mucin blood group substance solution varying in age from 1 to 5 days were clarified by Seitz filtration. Samples of each filtrate were added to an equal volume of 0.1 per cent mucin blood group substance in the basal salt solution at pH 7.0 and incubated under toluene at 30 C for 48 hr. As controls, samples of each filtrate, heated to 100 C for 15 min, were incubated with substrate in a similar manner. Hemagglutination inhibition assays (A anti-A) at the end of the incubation period showed that no destruction of A antigen had occurred in any of the mixtures, indicating the absence of an A-splitting enzyme in unheated culture filtrates.

In order to rule out the possibility that free enzyme had been removed from the cultures by Seitz filtration, 1- and 2-day-old cultures in mucin blood group substance solution, in which destruction of the A antigen had not gone to completion, were centrifuged in the cold to remove bacterial cells. The supernatant fluids were then incubated at 30 C under toluene for an additional 48 hr, along with samples of each heated to 100 C for 10 min. At the end of this additional period of incubation, no change in the content of blood group A antigen in these supernatant fluids was found to have occurred. Both heated and unheated supernatant fluids were proved to be sterile by subculture on nutrient, sheep blood, and mucin blood group substance agar.

Discussion

The coccobacillus of Chase (1938) proved able to metabolize polysaccharides of animal origin regardless of serological specificity. Growth was accompanied not only by destruction of any recognized antigens but also by degradation of each polysaccharide and its components. Intact polysaccharides were not required, since residues of mucin blood group substance partially degraded by mild acid hydrolysis and the dialyzable fractions resulting therefrom supported growth. Growth in salt solution containing N-acetylglucosamine as the only sugar was accompanied by a marked drop in N-acetylglucosamine color and reducing sugar values. On the other hand, no growth occurred in complete acid hydrolyzates of blood group substance nor in combinations of L-fucose, galactose, and glucosamine. All of these observations support the view that N-acetylglucosamine at least is essential for growth, whether as a part of a polysaccharide complex or uncombined. This would tend to explain the failure of this microorganism to metabolize polysaccharides lacking N-acetylglucosamine, or to grow on completely hydrolyzed blood group substance in which N-acetylglucosamine has been hydrolyzed to glucosamine.

Although the organism demonstrated a remarkable ability to degrade soluble blood group substances, growing cultures had no effect on A or B antigens of intact erythrocytes. The N antigen, however, was destroyed. In addition, after exposure to growing cultures or to resting bacterial cells, the intact erythrocytes became agglutinable by plasma or serum from the same donor (panagglutinable). The destruction of the N antigen and the development of panagglutinability may represent the effect of enzymes other than those which are responsible for the inactivation of A, B, and O(H) antigens.

It was established in the present study that culture filtrates and supernatant fluids were devoid of detectable blood group splitting enzymes. The enzymes responsible for destruction of the A, B, and O(H) antigens in cultures of blood
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Group substances must be assumed therefore to be primarily intracellular.

Summary

A coccobacillus originally isolated and described by Chase (1938) was investigated because of its ability to attack blood group substances. It was found that a number of purified substances of animal origin were metabolized by this microorganism in culture, whether detectable A, B, or O(H) antigens were present or not. Blood group A, B, and O(H) antigens were destroyed when included in the defined medium. N-Acetylglucosamine was essential for growth. Erythrocytes of blood groups A and B became panagglutinable but retained their specific reactivity with anti-A and anti-B, respectively, after incubation in cultures of the organism or in the presence of resting bacterial cells.

References


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