Almost everyone who has reared insects knows that it is very difficult to bring all to maturity. Some are infested with insect parasites; some die for a lack of proper food or living conditions; and others just die. Those that "just die" present interesting problems to some of us and consternation to others who are working out life histories. The summer of 1926 at the Station for the Study of Insects of The American Museum of Natural History in the Harriman State Park offered a number of cases of "just dying" and the bacterial causes of some of these deaths are reported here. On the other hand, not all bacteria living in insects kill their hosts. For examples, *Bacillus lasiocampa*, new species, seems merely to prevent females of the Tent Caterpillar Moth from laying their eggs, while *Proteus photuris* appears to be at most a normal symbiotic inhabitant of the luminous organ of fireflies.

Through the kindness and interest of Dr. Edward C. Rushmore, Superintendent of the Tuxedo Hospital, we were enabled to use their splendidly equipped laboratory in this study. It is impossible for me to state the amount of assistance afforded us in this work by Dr. Rushmore and his technician, Miss Margaret Stevens. The work could not have been done without their most valuable aid.

*Bacillus lasiocampa*, the organism that seemed to prevent the female Tent Caterpillar Moth (*Malacasoma americana*) from depositing her eggs, was found to be throughout the entire genital system and was readily grown from the dissected organs. This led to the plating of a number of egg masses to determine whether it occurred in eggs that might have been laid. We did not find it there. However, from one of these masses of crushed eggs a second organism was isolated in a pure culture, *Alcaligines stevensii*, new species.

In cooperation with Mr. William S. Creighton, a research guest at the Station, we were able to raise a culture of bacteria from agar slants.

1St. George's School, Newport, R. I.
inoculated with the luminous organs of the large common firefly, *Photuris pennsylvanicus*. Although a great number of experiments were carried on, none proved that these bacteria are responsible for the light. The organism isolated, *Proteus photuris*, is a member of a genus of extreme proteolytic action and so might be the source of luciferase, though at present we have no proof of such.

A sprig of asparagus to which were clinging a dozen or more dead and dying green flies, *Lucilia sericata*, was sent from Ramsey, New Jersey, to the Station by Mrs. Frank E. Lutz. At first we thought that the causative agent was the mycelium of a mold noticed on the decaying flies. Being interested, I cultured several flies that were in more or less advanced stages of decomposition, several that were dead but showed no signs of decay, and two that were dying. From the first group I derived a splendid growth of a mold of the genus *Aspergillus* and a very small colony of *Bacillus lutzae*, new species; from those that had just died, flourishing colonies of *B. lutzae*, *Micrococcus rushmorei*, new species, and *Neisseria luciliarum*, new species; and from those in dying condition, pure strains of *B. lutzae*. This seemed to indicate that the disease was caused by the bacillus rather than either of the cocci or the mold. In order to test it I made a set of broths inoculated with each organism and along with a sterile control put each under a tumbler with six to ten house flies. All the flies were dead in a few days including those in the control, most seemingly having drowned. Since then I have repeated the experiment with sufficient room for the flies to die outside the broth. I found that the bacillus was pathogenic to house flies and surmise that it was the pathogen in the case of the wood flies. Of ten imprisoned with the bacillus-broth three were dead on the fourth day and four more on the next. Of these, two in each batch yielded the organism. None of the dead in the other chambers yielded the organisms in question.

*Micrococcus flaccidifex danai*, new subspecies, was found to be the cause of the death of a Monarch Butterfly larva (*Danais archippus*) that was about to pupate. Possibly this is one of the reasons for the great decline in the numbers of this species of butterfly in the northeastern states during the past six years. One evening the larva was in ‘sound health’; the next morning it was hanging by its prolegs from the underside of a leaf, a black, flaccid object oozing with a black fluid. Smears showed this to be little more than body fluid teaming with motile cocci. One can not be certain from the meager cultural details given by Glaser and Chapman (Science, XXXVI, pp. 219–224) of *flaccidifex* whether the present organism is specifically distinct. On account of its pathological
effect it seems to be closely related to *flaccidifex* and I am naming it as a new subspecies. I failed to find any undue gyrating of the cocci in the hanging drop upon which they erect their genus *Gyrococcus*.

All of the experiments and cultures were prepared with rigorous observation of the rules of bacteriologic technique and all media used were prepared according to accepted standards. The Index Number is based in the scale adopted by the Society of American Bacteriologists at their December meeting, 1920.

**Neisseria luciliarum**, new species

**Index Number.**—2322-51220-2131

**Etiology.**—Derived from dead *Lucilia sericata* (*Diptera*) killed by *Bacillus lutze*.

**Morphology.**—A diplococcus varying in size according to the medium upon which it is grown. Stains Gram negative; absorbs pink from eosin-methylene-blue agar. On nutrient agar, gelatine, and in milk the individual cells are spheric slightly flattened on the adjacent sides. They vary from 0.5 microns on agar to 0.9 microns in diameter on milk. On Krumwiede’s triple sugar agar and on lead acetate agar the diplococci are typical "coffee-bean" forms ranging from 1.2 to 1.5 microns in diameter. It is a strict aerobe, motile, does not form spores or a capsule. Taxonomically it should be placed near *N. perflava* of Bergey *et al*.

**Biology.**—The organism is physiologically moderately active. It produces enzymes that ferment pentose and hexose monosaccharides, hydrolyze some disaccharides (sucrose) and weakly act upon some polysaccharides (glycogen and inulin). It digests gelatine; but little or no action takes place on casein. Nitrates are reduced. Litmus is reduced.

**Cultural Characteristics and Reactions**

**Nutrient Agar.**—Twenty-four hour stroke: there is a very scant growth; smooth, regularly margined; dirty white in color. Thirty days: whitish; irregular, very finely granular to smooth-surfaced colonies; margin with irregularly spaced incisions. Optimum 20-25 degrees C.

**Potato.**—In two days a thick, creamy, moist, cream-colored growth.

**Gelatine Butt.**—Nutrient not liquefied. A depressed colony starts growing immediately, slowly spreads over the surface, and works downward slightly widening the needle path and develops a typical napiform cavity. The depression is always about half full of a very viscous but not stringy, white bacterial sludge.

**Litmus Milk.**—Milk does not seem to favor a rapid growth of this organism. The litmus is reduced on about the seventh day. On the tenth day a slight separation of curd takes place but does not advance appreciably.

**Peptone Broth.**—Cloudy; no sediment, ring, nor pellicle.

**Triple-sugar (Krumwiede’s).**—Brilliant color develops about stab in twenty-four hours. Entire butt is colored in nine days and does not fade out within thirty-days.

**Hydrogen Sulphide.**—None in twelve days on lead acetate nutrient agar. This medium is unfavorable to good growth of bacteria.
Aldehydes (Endo's medium).—Negative for first twenty-four hours. Weakly positive in forty-eight hours strengthening with subsequent growth but never more than a weakly positive reaction.

Nitrate Broth.—Nitrates reduced to nitrites without the evolution of gas.

Diastatic Action.—None on starch nutrient agar or on starch broth.

Fermentation.—Carbohydrate | Gas | Broth | Sediment
---|---|---|---
Arabinose | x | Cloudy | Heavy
Dextrose | x | " | Flocculent
Galactose | - | " | Slight
Mannite | x | " | Flocculent
Lactose | 0 | " | Flocculent
Sucrose | x | " | Heavy
Starch | 0 | " | None
Inulin | - | " | None
Glycogen | - | " | Flocculent

Micrococcus rushmorei, new species

Index Number.—3332–5229/5–2333

Etioology.—Found along with Neisseria lucilia as a secondary in Lucilia sericata (Diptera) that had died of a disease caused by Bacillus lutæ.

Morphology.—A small Gram negative, staphyloid micrococcus culturally close to Micrococcus varians and aurantiacus. It is not motile. A capsule is generally present, sometimes enclosing two individuals which then appear like a small rod. It does not form spores. The cocci are largest on agar, measuring from 0.7 to 0.8 microns in diameter, on gelatine it is smallest, measuring from 0.2 to 0.3 in diameter. Diplococci, triads and tetrads are not uncommon. It is a facultative aerobe.

Biology.—M. rushmorei is inactive as an enzyme producer. None of the carbohydrates produce gas with it. Gelatine is slowly digested. It has no effect on milk. Aldehydes are formed in good quantity. Nitrates are reduced. Litmus is reduced.

Cultural Characteristics and Reactions

Nutrient Agar.—Twenty-four hour stroke is an extensive, thin, foliate margined, growth with much indistinct secondary lobing and is pigmented faintly yellow. Thirty day very finely granular, margin scalloped deeply and deeply cut by radial sutures; extreme margin faintly white to pale peach-yellow, and darker inner band of chrome yellow. The original colony was peach-colored.

Potato.—A medium thick, very dry, granular, pale peach-yellow growth.

Gelatine Butt.—No liquefaction in twenty days. Growth very slow and napiform. The lower part of the chamber is filled with a very viscous bacterial sludge.

Litmus Milk.—No coagulation in fifteen days. Litmus reduced on the seventh day. Reaction neutral throughout.

Peptone Broth.—Clear; no sediment, ring, nor pellicle.

Triple Sugar (Krumwiede's).—Completely and deeply colored in five days, first color appearing in twenty-four hours. In twelve days the color begins to fade and is entirely gone thirty days after the inoculation.

Hydrogen Sulphide.—None formed.
NEW BACTERIA FOUND IN INSECTS

Aldehydes (Endo's medium).—Very strongly positive reaction in twenty-four hours.

Nitrates.—Nitrates reduced to nitrites without the evolution of gas.

Diastatic Action.—None as indicated on starch nutrient or broth.

<table>
<thead>
<tr>
<th>Fermentation.</th>
<th>Carbohydrates</th>
<th>Gas</th>
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<tr>
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<td>Very slight cloud</td>
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</tr>
<tr>
<td>Inulin</td>
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<td>Very slight cloud</td>
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</tr>
<tr>
<td>Glycogen</td>
<td>0</td>
<td>Clear</td>
<td>Slight</td>
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</tbody>
</table>

Micrococcus flaccidifex danai, new subspecies

Index Number.—3322-22120-2131.

Etiology.—Caustive agent of "wilt" disease of the Monarch Butterfly (Danais archippus).

Morphology.—A minute coccus, motile, staining Gram negative. In artificial cultures it often forms small rod-like diplococci, none of this form were noted in the direct smears. It does not form spores. The presence of a capsule was only doubtfully demonstrated. The coccus varies between 0.1 and 0.2 microns in diameter on most media to 0.5 microns in lead-acetate agar and in the direct smears. On nutrient agar a staphlo-like growth is not uncommon and triads and tetrads are found in most media. The flaccidifex group seems to be culturally near the freudenreichii group. A facultative aerobe.

Biology.—It is very active in the fermentation of carbohydrates. Gas is produced with all monosaccharides used. An invertase acts on sucrose and produces gas. Soluble polysaccharides are fermented with the formation of gas. Gelatine and casein are slowly digested. Aldehydes are produced and nitrates are reduced.

Cultural Characteristics and Reactions

Nutrient Agar.—Twenty-four hour growth is a shining dirty white colony with an incised crenate margin. A thirty day colony is white, smooth and thin, the edge is undulating and the marginal zone quite transparent and is finely cut by radii. Oblique transmitted light causes a very marked opalescence.

Potato.—A thick, creamy, dry growth with a crenated surface.

Gelatine Butt.—Gelatine is liquefied and in part digested with the formation of a heavy bacterial sludge. In the first stages the liquefaction is napiform and changes to infundiform with age. A ring is left on the test tube wall but no pellicle is formed.

Litmus Milk.—Slightly acid on the second day, no coagulation until the third day. The casien is slowly but not completely digested, there is no reduction of the litmus.

Peptone Broth.—Cloudy; no sediment, ring, nor pellicle.

Triple Sugar (Krumwiede's).—Color developes in twenty-four hours and is retained at least thirty days.
HYDROGEN SULPHIDE.—None formed.

Aldehydes (Endo’s medium).—Negative the first day, a weak reaction the second day and a “plus-minus” reaction at the end of four days.

Nitrates.—Nitrates are reduced to nitrites, no gas evolved.

Diaspatic Action.—None.

Fermentation.—Carbohydrate | Gas | Broth | Sediment
---|---|---|---
Arabinose | x | Cloudy | Flocculent
Dextrose | x | Cloudy | Flocculent
Galactose | | Clear | Slight, heavy
Mannite | x | Cloudy | Slight, heavy
Lactose | 0 | Slightly cloudy | Slight
Sucrose | x | Cloudy | Heavy
Starch | 0 | Cloudy | Slight, heavy
Inulin | x | Cloudy | Heavy
Glycogen | x | Cloudy | Flocculent

Alcaligines stevensae, new species

Index Number.—53?2-21230-2333

Etiology.—Found as a pure culture in the eggs of a Tent Caterpillar Moth (Malacasoma americana) constituting an irregular and poorly formed egg-mass.

Morphology.—A small variable rod probably allied to A. bronchisepticus. It stains Gram negative; forms neither spores nor capsule and is very motile. On agar the organism measure about 0.2 by 0.6 to 0.8 micron in size. In broth, particularly sugar broths, they grow to extraordinary length, 0.3-0.5 by 3.0-3.5 microns. Transplants from broth to nutrient agar are normal size in six hours. On triple-sugar medium the form varies from “square coccoids” of 0.2 microns to rods up to 0.2 by 0.8 microns. Chains and mats are rarely met with. It is an aerobe.

Biology.—None of the carbohydrates are fermented by this organism. In all other respects too it is rather inactive. Gelatine is not digested, milk is unaltered or only slightly affected. No reduction takes place. Aldehydes however are produced in fair amount.

Cultural Characteristics and Reactions

Nutrient Agar.—Very slow growth at all temperatures. Twenty-four hour stroke either punctiform or a weak grayish shining growth with a smoothly undulating margin. Thirty-day growth same as twenty-four hour except for a very light gray-brown color in the middle.

Potato.—In two days there is a fairly thick, pale straw shining growth.

Gelatine Butt.—No liquefaction or digestion in nine days. A weak surface growth.

Litmus Milk.—Neutral, no reduction, a slight separation of curd on the seventh day.

Peptone Broth.—Clear; no sediment, ring, nor pellicle.

Triple Sugar (Krumwiede’s).—Grows well on this medium. Color appears in twenty-four hours and increases to a maximum in fourteen days. On the eighteenth day it begins to fade but never becomes fully decolorized.

Hydrogen Sulphide.—None generated.
NEW BACTERIA FOUND IN INSECTS

NEW BACTERIA FOUND IN INSECTS

ALDEHYDES (Endo's medium).—Very weakly positive in twenty-four hours, becoming a “plus-minus” in forty-eight hours and decidedly positive in three days.

NITRATES.—No reduction in four days.

DIASTATIC ACTION.—None on starch agar or in starch broth.

FERMENTATION.—Carbohydrate Gas Broth Sediment
Arabinose 0 Slightly cloudy Flocculent
Dextrose 0 Cloudy Slight
Galactose -0 Clear None
Mannite 0 Clear None
Lactose 0 Clear None
Sucrose 0 Cloudy Slight
Starch 0 Clear None
Inulin 0 Slightly cloudy Slight
Glycogen 0 Slightly cloudy Slight

Bacillus lasiocampa, new species

INDEX NUMBER.—5121-21120-2333

ETIOLOGY.—This bacillus was found in the ovaries and egg tubes of the Tent Caterpillar Moth (Malacasoma americana). The females so affected seem to be unable to deposit their eggs.

MORPHOLOGY.—A member of the subtilis group. It is large, stains Gram positive and is aerobic. Young specimens stain uniformly, older sporulating individuals stain polarly. It does not stain acid fast. It is sluggishly motile. In agar the size ranges from 0.8 by 2.5 microns to 1.0 by 3.0 microns: spores are elliptic 0.8 by 1.2-1.8 microns.

BIOLOGY.—Unable to ferment carbohydrates but has a strong proteolytic action, gelatine and milk are rapidly digested. Nitrates are reduced. Litmus is reduced.

CULTURAL CHARACTERISTICS AND REACTIONS

NUTRIENT AGAR.—Growth on agar rapid and heavy, with a characteristic form. In twenty-four hours from the original stroke “fans” radiate along the entire length, these gradually increase in size and become confluent. The outer edge of the growth is of a moist appearance and with little color while the older parts are dry and dirty white. Cultures of four days and older show a marked “zoning” or what appears upon examination to be concentric rings of spore-bearing individuals. Thirty-day cultures have a very strongly wrinkled surface with no defined system to the ridges. From the center of the colonies there are groups of fine fibrous crystals extending down into the agar.

POTATO.—A heavy, thick, dry, white to gray-white growth.

GELATINE BUTT.—Ten cc. liquefied completely in four days, a heavy tough pellicle formed and a flocculent precipitate filling the lower half of the fluid.

LITMUS MILK.—Completely decolorized on the second day and a very fine coagulation formed. This curd is digested rapidly and on the seventh day is almost entirely done away with.

PEPTONE BROTH.—Clear; no sediment, a heavy brittle pellicle.

TRIPLE SUGAR (Krumwiede’s).—Color appears in twelve hours and is well developed in twenty-four hours. It begins to fade noticeably by the seventeenth
day and is usually entirely gone at the end of thirty days. Slants fade out more rapidly, clearing in twenty-one days.

HYDROGEN SULPHIDE.—None, as shown on lead-acetate agar.

ALDEHYDES (Endo’s medium).—None formed.

NITRATES.—Nitrates reduced to nitrites without the formation of gas.

DIASTATIC ACTION.—None on nutrient starch agar or in starch broth.

Fermentation.—Carbohydrate Gas Broth Sediment

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Gas</th>
<th>Broth</th>
<th>Sediment</th>
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</thead>
<tbody>
<tr>
<td>Arabinose</td>
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<tr>
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<td>Glycogen</td>
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</tr>
</tbody>
</table>

Bacillus lutze, new species

INDEX NUMBER.—5212-21135-2313

ETIOLOGY.—The organism seems to be pathogenic to some flies. The material was derived from dying and dead Lucilia sericata (Diptera) thought to be infected with a muscod disease. Dying individuals and those just dead but showing no sign of decay yielded pure cultures. Those in decay yielded a mycelium and the two cocci described.

MORPHOLOGY.—A rather small “cocco-bacillus,” probable of the same group as B. albus liquifaciens of Klaman. Stains Gram negative. It is polarly spore-bearing and very motile, probably by means of peritrichous flagella. In regard to Gram’s method of staining it has a very characteristic habit. Very young coccoid forms on agar—three to six hours old—stain positive, cultures up to twelve hours old generally stain amphophile and in all older growths both the coccoids and rods stain clearly negative. In the media the forms vary considerably. Gelatine yields pure rods 0.5 X 2.0 microns; agar and sugar broths have the rods greatly in predominance, many of them short but very few coccoids; triple sugar medium favors the very short rods and coccoids; lead-acetate agar fosters a purely coccid strain with a few pairs. These coccoids are not spheric but rather squared and measure from 0.5 to 0.9 microns in diameter. In litmus milk all forms are present and it is not uncommon to find chains of individuals of all phases.

BIOLOGY.—B. lutze is inactive in the fermentation of carbohydrates, lactose is the only one with which gas is produced. It has a weak proteolytic action. It does not form aldehydes or reduce oxygen salts.

CULTURAL CHARACTERISTICS AND REACTIONS

NUTRIENT AGAR.—Twenty-four hour stroke: bright golden yellow, glistening, rounded colonies with no characteristic emargination. Thirty days: same as twenty-four hour except that the margins are very finely crenate and incised.

POTATO.—Medium thick, rather moist and soft, glistening rich yellow growth.
NEW BACTERIA FOUND IN INSECTS

GELATINE BUTT.—In twenty-four hours a shaft is formed along the needle path, this increases in size and a flocculent growth appears at its bottom. The gelatine is soft but not liquefied until the eighth day. Twenty-four hours after liquefaction a thick tough pellicle is formed. In thirty-three days 10 cc. of medium was liquefied. A deposit of bacteria is always at the bottom of the well.

LITMUS MILK.—No reaction for fourteen days, then a slight acidulation and partial reduction of the litmus.

PEPTONE BROTH.—Cloudy, much heavy sediment, a tough membranous pellicle.

TRIPLE SUGAR (Krumwiede’s).—Faint color after twenty-four hours. Entire tube richly colored in four days. Starts to fade in fourteen days but does not completely lose its color.

HYDROGEN SULPHIDE.—None formed.

ALDEHYDES (Endo’s medium).—None formed.

NITRATES.—No reduction.

DIASTATIC ACTION.—None on starch nutrient agar or starch broth.

Fermentation.—Carbohydrates Gas Broth Sediment
Arabinose 0 Slightly cloudy Heavy
Dextrose 0 Cloudy Heavy
Galactose 0 Flocculent Heavy
Mannite 0 Cloudy Slight
Lactose x Flocculent Flocculent
Sucrose 0 Slightly cloudy Heavy
Starch 0 Cloudy Heavy
Inulin 0 Slightly cloudy None
Glycogen 0 Slightly cloudy Heavy

Proteus photuris

INDEX NUMBER.—5332-42120-2131

ETIOLOGY.—Derived by Mr. William S. Creighton from the luminous organs of the firefly (Photuris pennsylvanica). MorPHOLOGY.—A minute, Gram negative rod, microscopically resembling para-typhoid B, but slightly smaller. It is not motile nor does it form a capsule. Some specimens seem to stain polarly but repeated trials with Neisser’s Stain fail to demonstrate spores. On agar the organism measures 0.3 x 0.5 microns. They are larger in broths, 0.3 x 1.0 microns. Very poorly facultative aerobe in some strains, purely aerobic in most cases.

BIOLOGY.—The lower saccharides are freely fermented (except lactose) with the formation of gas. Inulin weakly produces gas. Gelatine is rapidly digested but casein is unaffected. Aldehydes are produced. Nitrates are reduced.

CULTURAL CHARACTERISTICS AND REACTIONS

NUTRIENT AGAR.—Twenty-four hour stroke: smooth, thin, dirty-white, wet appearing colonies; the edges are irregular or with angular projections. Fourteen day old cultures show a pale yellow center that at the end of a month darkens to a pale brown. Thirty day colonies are very irregular, amoeboid, slimy growths, white at the margin and yellowish to light brown in the middle. A mesh of heavy threads is located centrally. A number of fine needle-like crystals extend into the medium from the middle of the colonies.
Potato.—In two days a very thick, creamy, pale straw to dirty white, wet appearing growth.

Gelatine Butt.—Liquefaction sets in immediately and 10 cc. is liquefied in nine days. No ring or pellicle is formed. A powdery bacterial sediment is present. Extremely putrid odor.

Litmus Milk.—No change in acidity. Coagulation starts on the second day with the separation of a very fine curd. No digestion at the end of twenty days.

Peptone Broth.—Cloudy; light brittle pellicle, heavy sediment, heavy tenacious ring.

Triple Sugar (Krumwiede's).—Color develops slowly; twenty-four to thirty-six hours pass before any noticeable change starts. It increases for seven days after the inoculation but never attains a very brilliant hue. Suddenly on the eighth day the color entirely disappears. This reaction is characteristic and has been checked repeatedly. There is no subsequent color development though a luxurious growth of bacteria follows.

Hydrogen Sulphide.—None generated.

Aldehydes (Endo's medium).—A very weak reaction in twenty-four hours gradually strengthening to a very positive one in three days.

Diastatic Action.—None on starch nutrient agar or on starch broth.

Fermentation.—Carbohydrates Gas Broth Sediment

<table>
<thead>
<tr>
<th>Carbohydrates</th>
<th>Gas</th>
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<tbody>
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**Fermentation**

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<th>Mannite</th>
<th>Lactose</th>
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