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KONINKLIJKE AKADEMIE VAN WETENSCHAPPEN
TE AMSTERDAM.

PROCEEDINGS OF THE MEETING
of Saturday January 30, 1909.

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Microbiology. — *"The splitting up of ureum in the absence of albumen."* By Dr. N. L. SÖHNGEN. (Communicated by Prof. S. HOOGEWERFF).

(Communicated in the meeting of October 31, 1908).

§ 1. *General considerations. Ureum as a source of energy.*

Ureum, secreted as a product of the katabolism in the higher organized animal world, leaves the body, dissolved in urine.

As such this nitrogen-compound cannot be assimilated by the higher vegetable world, and hence it would be of no practical importance for us, if there were no fungi, especially certain microbes everywhere in the ground, which changed it into assimilable compounds.

It is for this reason that we have to consider urine, more particularly ureum, as one of the most valuable sources of nitrogen for arable land.

Millions of kilogrammes of the indispensable nitrate-nitrogen are annually in a biological way formed from urine in the ground and are of the greatest use to vegetation.

Nitrogen taken by man and animal as vegetable albumen, leaves the body again for the greater part in the form of ureum, and in this way describes a cycle.

A rough calculation of the quantity of ureum, which in our country is produced by the population and the cattle, gives an idea of the enormous quantity of nitrogen describing this cycle.

The data for the amount of cattle have been taken from *Verslagen en Mededeelingen van de Directie van den Landbouw.* (Reports and Communications of the Board of Agriculture).

The quantity of ureum, daily secreted by the population, amounts to ± 125000 K.G.; by the cattle ± 225000 K.G., making ± 350000 K.G., or ± 350 tons a day.

By biological oxidation, a quantity of nitrate-nitrogen would be produced equal to that found in ± 900 tons of saltpetre.

Annually from the ± 125000 tons of ureum formed, ± 350000 tons of saltpetre could be produced, representing a value of ± 3.5 millions of £. sterling and this, distributed over the 2155000 acres of arable land, would yield ± 160 K.G. of saltpetre pro acre.

That only a trifling part of this enormous mass is utilized for agricultural purposes, need not be proved here. Especially in large towns for hygienic reasons almost all ureum is lost to any useful purpose; on the other hand it would decidedly be of great value for farms in the country, to be more careful about collecting urine.

The above mentioned considerations may serve to draw once more attention to the enormous value represented by ureum as a manure.

In the experiments about microbes decomposing ureum the culture media generally were characterized by the presence of albumen and peptones.

It is true that VON JAKSCH [1]) and BEIJERINCK [2]) have made experiments with salts of organic acids as a source of carbon, but a systematic investigation in this direction has not been made as yet.

VON JAKSCH's investigation in 1881 was especially of importance for

[1]) Zeitschrift für Phys. chem. 1881.

[2]) Centralbl. f. Bakt. II, Abt. VII, Bd. 1901.

the study of the conditions of nutriment of ureum-bacteria. It taught us that carbo-hydrates, salts of fatty acids and of organic multibasic acids can be assimilated.

The so highly interesting studies of BEIJERINCK about the decomposition of ureum by microbes principally treat of the ureum-decomposing organisms which in cultures, on application of his *accumulation-method* in bouillon 10 °/₀ ureum make themselves conspicuous. In the course of the investigation some experiments have been made with culture-liquids, composed of water, 5 °/₀ ureum, 0.025 K_2HPO_4 and 1 °/₀ ammoniumoxalate, natriumacetate, seignette-salt, ammoniumcitrate and ammoniummalate. In these culture-media a strong decomposition of ureum takes place after infection with mould.

The 5 °/₀ ureum added, however, are not entirely decomposed. The easily assimilable compounds, such as ammoniummalate and citrate, give rise to a greater ureum-decomposition, respect. 4°/₀ and 3°/₀, than those which are not so easily assimilated, such as ammoniumoxalate and natriumacetate, in whose presence only 2 °/₀ ureum is decomposed.

The study of the microbes which are found in these cultures, was not continued at that time.

The purpose of this investigation is therefore principally to prove that the life of numbers of ureumbacteria is by no means dependent on the presence of albumen, but that for these ferments the large quantities of carbo-hydrates and salts of organic acids, which for microbial life are available in mould are extremely fit as a source of carbon, whilst at the same time the ureum can serve as a source of energy as well as as a source of nitrogen.

Some preliminary experiments led to the conviction that the most different sources of carbon, in culture-liquids containing these compounds and ureum, dissolved in water, 0,05 °/₀ K_2HPO_4 are excellently fit for the growth of weak as well as for very strong ureum-splitting microbes.

Cultivated in a thin layer of liquid in Erlenmeyer-recipients at ± 33°, being the optimumtemperature of the growth, strong species, especially those producing spores appear; at a low temperature, 15°—23°, less strong splitting ferments, especially micrococci are produced.

The exclusion of other groups and the privilege of the ureum-bacteria in these culture-media is so complete, that the latter mixed with raw materials, such as mould, sewer or ditchmud, after some days contain only ureumsplitting organism.

If one of these cultures, infected with raw material, is put into sterilised liquids of the same composition, the ureum-fermentation also progresses very well there.

Which ureum-splitting species will appear depends upon the composition of the source of carbon added and the degree of alkalinity of the culture-medium.

In § 2 and § 3 we shall revert to this more in detail.

Ureum as a source of energy.

Ureum gives to the ureum-splitters exclusively energy; in no circumstances whatever it is fit to serve at the same time as a source of carbon.

Different experiments which I have made about this, corroborate the truth of former investigations; neither can ureum serve as oxidizable material in the sulphate-reduction; denitrification with ureum is also impossible.

The part that ureum plays in the growth of microbes, is therefore sharply determined. Always the presence of some suitable source of carbon is necessary; this carbon-compound is partly oxidated and therefore also this part serves for energy, partly it is assimilated.

For the above-mentioned oxidation of the source of carbon atmospheric oxygen is used; the quantity necessary is very small, which can be proved by cultivation in bottles with a stopper, which are entirely filled with the culture-liquid.

Only the oxygen dissolved in the culture-liquid is then available for the microbes and nevertheless the ureum-splitting then takes place just as well as when the supply of oxygen is abundant.

If, however, the culture-liquid has previously been made free from oxygen by boiling, after infection no ureum-splitting takes place in a bottle completely filled.

From these experiments follows that a good ureum-splitting is possible, while only very little organic matter is oxidated.

Now it is a fact that on the whole strong splitting ferments show in the cultures a very slight growth and from this it follows that also the quantity of carbon, necessary for the structure of bacterial bodies is very small.

These facts provè that a small quantity of a suitable organic compound, in the presence of ureum, must be sufficient for a complete development of the organisms and a normal ureum-decomposition.

Now, in order to ascertain what part of the sum of energy developed in the culture, is developed in the splitting up of the ureum the minimum quantity of carbon-compound, sufficient for a normal ureum-decomposition and growth, was determined. For this purpose experiments have been made with the afterwards described *Bacillus*

erythrogenes and *Urobacillus jakschii* in series of culture-liquids, which, besides ureum, contain a diminishing quantity of asparagina or ammoniummalate.

Indeed a very trifling quantity of these materials proves to be sufficient for a normal ureum-decomposition.

From the results of the investigations, laid down in the subjoined table, it follows that the *Bacillus erythrogenes* at a normal growth splits 500 mG. of ureum with 20 mG. of carboncompound whilst the *Urobacillus jakschii* splits 1800 mG. of ureum with 10 mG. of carbon-compound.

With smaller quantities of carbon-compound the growth of both microbes is considerably less than above.

The quantity of energy, which in the *erythrogenes*- and *jakschii*-cultures was developed by the splitting of the ureum, amounts respectively to ± 96 % and 99 % of the total sum of energy developed in these cultures.

At the same time it appears from these numbers that the less splitting species want a larger quantity of carbon-compound for the decomposition of a certain quantity of ureum than the very strong splitters.

The figures in the subjoined table denote the number of c.c. N H_2SO_4, necessary for neutralizing 50 c.c. culture-liquid after five days of culture at a temperature of 30°.

The 50 c.c. culture-liquid inocculated with the *Bacillus erythrogenes,* consist of water, in which 0.05 % K_2HPO_4 2 % ureum and the carbon-source are dissolved.

The 50 c.c. culture-liquid infected with *Urobacillus jakschii* has, besides 5 % ureum instead of 2 % ureum, the same composition as the above mentioned.

Decomposition by Bacillus erythrogenes.

Quantity of carbon source in milligrammes	50	40	30	20	10	5
Decomposition if the latter is asparagine	18.5	17.5	17	17	13	8
Decomposition if the latter is amm. malate	19.8	17.9	18.5	18.0	14.2	9.5

Decomposition by Urobacillus jakschii.

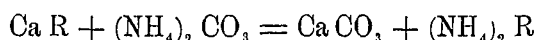
Decomposition if the latter is asparagine	61.5	60	59	60	54	42
Decomposition if the latter is amm. malate	60	58	60	59	56.5	39

§ 2. *Calciumsalts of organic acids as a carbonsource for ureum-splitting microbes.*

The organic acids proceeding from plants or produced by fermentation thereof are principally neutralized in arable soil by the frequently occurring calciumcarbonate.

The general occurrence, therefore, of these salts in the soil caused, for the following investigations, calcium-compounds of organic acids to be chosen in the first place as a source of carbon for ureum-splitting bacteria.

If in a culture-liquid, containing these salts and ureum, dissolved in water to which 0.05 K_2HPO_4 is added, ureum is split, the ammonium-carbonate formed will not directly bring about a considerable increase of alkalinity of the medium, but in the first place it will be neutralized by the calciumsalt and that according to the formula:



$$Ca\,R + (NH_4)_2\,CO_3 = Ca\,CO_3 + (NH_4)_2\,R$$

Therefore the calcium-compounds of the organic acids exercise a retarding influence on the alkalinity; for not until all the calcium is united with the carbonic acid formed, the alkalinity will advance rapidly; then the culture-liquid is like one that contains an ammoniumsalt of an organic acid as source of carbon. This is treated of in § 3.

But it is especially because of the existence of this period of rest before the increase of alkalinity, that cultures with calciumsalts of organic acids are so particularly fit for the accumulation of less strong splitting organisms, by which means every opportunity is afforded for study of these kinds, which are otherwise so rapidly supplanted.

The cause that during this first period also the ureum-splitters have the advantage of all the microbes contained in the raw infection-material, so that the latter are already then entirely supplanted is that their specific source of energy, the ureum, is at their disposal.

So if we want to get an insight into the numerous kinds of weak ureum-splitters, we have to make a plate-culture before all calcium is united with carbonic acid.

As a rule a good result is obtained when after 2 or 3 days the plate-making takes place on meat-gelatine or on a culture-medium, composed of water, 10 °/₀ gelatine, 0.05 °/₀ K_2HPO_4, 0.05 NH_4Cl and 0.5 °/₀ calc. malate.

These experiments give a fair idea of the great number of ureum-splitting microbes which the soil contains; they follow each other as the process proceeds and as the alkalinity increases, till at last the strongest, the most powerful hydrolysing kinds are left.

To give a detailed description of the many weak ureum-splitting kinds that exist, would be of hardly any use.

During my experiments in October, November, and December 1904 in the *Microbiological Laboratory*, under the guidance of Prof.

BEIJERINCK at Delft, there regularly occurred in these cultures a microbe which drew the attention by the formation of a red and yellow colouring-matter on meat-agar and meat-gelatine. The colonies are of a bright yellow colour, whilst a red colouring-matter diffuses in the culture-plate.

This bacterium shows itself more especially in cultures with citrate and tartrate in large numbers, so that in using these salts the above mentioned species can be obtained in great numbers.

If in the same culture-liquid inocculation is repeated twice or three times at a temperature of 23° and a titre of ± 35 c.c. N: per 100 c.c. culture-liquid, this bacterium is often accumulated almost to pure culture.

Description of Bacillus erythrogenes.

Bacillus erythrogenes are among the very strong oxidating ferments; both sugars and salts of organic acids and also albumen are assimilated. In tap water 0.05 °/₀ $K_2 HPO_4$ a fair growth takes place in the presence of ureum, if one of the following compounds as a carbon-source is added: glucose, maltose, cane-sugar, asparagine, calcium- and natriumsalts of the volatile fatty acids (except of formic acid, which gives a slight growth) and the multibasic acids, such as apple acid, lactic acid, lemon acid, argol acid and amber acid (except oxalic acid).

Milk appeared to be a very suitable culture-medium. The development herein is attended by the appearance of a disagreeable sweet smell.

Even calciumhumate added as a carbon-source causes growth and therefore ureum-splitting.

Amylum, however, is not affected, so that evidently no diastatic enzym is formed.

The yellow colouring-matter belongs to the body of the bacteria and arises independent of the composition of the medium; however for its formation the influence of light is necessary.

The red, diffusing colouring-matter arises only in case the feeding takes place with albumen and the light is excluded. Arisen in the dark, this colouring matter will soon be decomposed when exposed to the light.

Cultivated on meat-agar while light is excluded, the white colony shows itself on a fine red diffusion-field; cultivated in the presence of light, there arises a bright yellow colony on a colourless field.

What influence the two colouring matters have on the vitalfunctions of the microbe, could not be stated.

Gelatine is melted by the strong splitting varieties, not by the weak ones.

The length of the bacterium amounts to 2—4 μ
Breadth 1—1.5 μ

The bacterium is endowed with the power of motion, and in liquids mostly occurs as a double bar; on solid media it sometimes forms strings.

No formation of spores takes place.

The optimum of the growth lies near ± 30°.

The optimum of its urease near ± 51°.

Ureum-splitting by the strongest species is found in the subjoined table.

The figures denote the number of c.c. $^1/_{10}$ N. H_2SO_4, which are necessary for the neutralization of 10 c.c. culture-liquid.

The culture has taken place at 43°.

In bouillon with ureum.

After days	1	2	3	4.
2°/₀ ureum	13.5	33.	45	44.
6°/₀ ureum	19.	45	68	68.

If we compare the species described here with those isolated by Lohnis [1]), they prove to agree in size and formation of a double colouring-matter; striking is the difference in the power of splitting ureum.

In his experiments a *bacillus erythrogenes* split in bouillon 2°/₀ and 5°/₀ ureum resp. $^1/_{10}$ °/₀ and 1°/₀ ureum, whilst the one described here splits in bouillon 2°/₀ and 6°/₀ ureum resp. $1^1/_3$ °/₀ and 2°/₀.

The less strong species, isolated here, still split in the culture-liquids named $^1/_2$ °/₀ and 1 °/₀ ureum respectively.

So it is clear that the species *Bacillus erythrogenes* includes varieties of very different ureum-splitting power.

The powerful splitters are at the same time characterized by the possession of tryptic-enzymes.

§ 3. *Ammoniumsalts of organic acids as a carbon-source for ureum-splitting microbes.*

Ammoniumsalts of organic acids are in media, which at the same time contain ureum and anorganic salts, superior to any other compounds for the development of strong ureum-spliting microbes.

Both the split ureum and the ammoniumcarbonate of the oxidated

[1]) Centr bl. f. Bakt. Abt. XIV Bd 1905.

ammoniumsalt that has become free contribute to the rapid rise of the alkalinity of the culture-liquid.

Provisional experiments proved that with a ureum-quantity of ± 5 % in these cultures the best results could be obtained; with this ureum concentration growth is still very good.

In a way quite analogical with the ammoniumsalts behave different sugars as carbon-sources for ureumsplitters; the species which are most remarkable generally agree with the powerful species isolated by MIQUEL.

A culture-liquid consisting of:

100 water

0.05 K_2HPO_4

1 ammoniummalate

5 ureum

infected with ± two gr. of mould or sewage and cultivated at ± 33° contains after 48 hours, sometimes even after 36 hours only ureum-splitting ferments. A total supplanting of all other organisms has taken place in that short time.

The decomposition of the ureum takes place in presence of easily assimilated carbonsources, such as malate and lactate, more rapidly than with compounds which are not so easily assimilated such as tartrate or acetate.

Malate is also for these organisms an exceedingly easily assimilable compound, as is generally the case; lactate, citrate, succinate, tartrate, butyrate and acetate follow next.

When, however, in a culture with one of these salts the final titre has been reached, the same powerful species are on the whole observed in malate as well as in tartrate and acetate cultures.

Now if we examine the sorts succeeding each other in these culture-liquids, it appears that, when sown upon meat-gelatine $^1/_2$ % ureum or ammoniummalate-gelatine $^1/_2$ % ureum, already after two days, when the titre is ± 60 c.c. N. per 100 c.c. culture-liquid, the number of micrococcis and melting bacteria rapidly diminishes; whilst the alkalinity increases, bacteria forming spores together with a ureum-splitter not forming spores take their place.

The many weak splitting organisms observed in the cultures with calciumsalts rapidly die off.

After 3 or 4 days only very strong hydrolysing microbes are left, whilst micrococcis and melting species have disappeared.

The growth on neutral meat-gelatine of the species found in strongly alkaline liquids is very slight or does not succeed at all.

In general the colonies on meat-gelatine 1 °/₀ ureum or ammonium malate-gelatine 1 °/₀ ureum are characterized by their small dimensions, whilst a field of calciumphosphate- and calciumcarbonate-crystals surrounds them.

After 5 or 6 days the titre has risen to a maximum of ± 125 c.c. N. per 100 c.c. of culture-liquid, so that ± 4 °/₀ ureum has been split.

The 4 or 5 species present are the *Urobacillus leubii* (BEIJERINCK) and the most powerful species described by MIQUEL, the *Urobacillus maddoxii*, *freudenreichii* and *duclauxii* together with a species not yet described and not forming spores, which will be called *urobacillus jakschii*.

After infection of cultures with ammoniummalate it is especially the *Urobacillus maddoxii* and *urobacillus duclauxii* together with the *Urobacillus jakschii* which predominate. Sometimes the *Urobacillus jakschii* supplants the two other species almost entirely and is almost accumulated to pure cultivation.

If we start from pasteurized material, it is especially the *Urobacillus maddoxii* and *Urobacillus duclauxii* which make themselves conspicuous.

In these culture-liquid the *Urobacillus pasteurii* BEIJERINCK did not occur, so that the latter may be said to belong to the ureum-bacteria which positively want albumen for their growth.

For the description of the *Urobacillus leubii*, *freudenreichii*, *maddoxii* and *duclauxii* it is sufficient to mention the chief characteristics.

The *Urobacellus jakschii*, however, will be described more in details.

Urobacillus leubii (BEIJERINK).

Urobacillus leubii, which generally occurs in the *Vorflora* of BEIJERINCK's accumulation-experiments, is a little moving bar which can get oblong spores.

On meat-gelatine with ammoniumcarbonate it is difficult to distinguish this species from *urobacillus pasteurii*. Inocculated from this medium on neutral meat-gelatine it grows into two species of colonies: viz. into yellow, troubled, thin colonies forming spores and into glassy, transparent colonies free from spores.

The growth is, however, upon meat-gelatine with ammoniumcarbonate much better than upon neutral meat-gelatine.

The spores can bear boiling heat and can be dried.

Gelatine is not melted.

In bouillon 6 °/₀ ureum 2½ °/₀ ureum is split in 4—5 days

Urobacillus freudenreichii MIQUEL.

Urobacillus freudenreichii is a little moving bar, 5—6 μ in length, 1 μ broad; on a firm medium it grows into long threads.

Elliptic glittering endospores are formed, which can stand a heat of 94° for two hours.

Neutral gelatine is slowly melted by the irregularly formed colonies, whilst gelatine to which ureum has been added, is not melted and the colonies on it assume the characteristic globular form.

2 °/₀ ureum in bouillon are decomposed within 4 days at 30°—35°.

MIQUEL isolated this species out of air, riverwater, soil and from the excrements of ruminants.

Urobacillus maddoxii MIQUEL.

A little moving bar, 3—6 μ long, 1 μ broad, forming oval endospores, which are able to bear a heat of 94° for two hours.

On neutral meat-gelatine it does often not develop, on ammoniacal gelatine the growth is rather good.

Within 3 days 2 °/₀ ureum in bouillon is split.

The bacterium has been isolated from sewage and river-water.

Urobacillus duclauxii MIQUEL.

Like the two preceding species moving, length 2—10 μ, breadth 0,6—0,8 μ.

The bacterium forms small elliptic endospores which are able to bear a heat of 95° for 2 hours.

In a neutral medium no growth arises, on ammoniacal meat-gelatine or on meat-gelatine provided with ureum there arise very small hardly observable colonies which are surrounded by crystals.

The gelatine does not melt, but it becomes like viscous after 40—50 days.

2°/₀ ureum in bouillon are decomposed within 24 hours.

Urobacillus jakschii.

Urobacillus jakschii is a small quickly moving bar in a culture-medium that is not too alkaline; if some percents of the ureum in it have been split, the motion stops.

Length of the bacterium 5—7 μ; breadth 1—1.5 μ. Spores are not formed.

On neutral meat-gelatine growth is seldom obtained; if, however ammoniumcarbonate or 1 $^0/_0$ to 2 $^0/_0$ ureum is added, there arise small coli-like colonies, surrounded by a wreath of calciumphosphate crystals.

The gelatine is not melted, but after a month, it is viscous.

2 $^0/_0$ ureum in bouillon are split in 24 hours. Of 10 $^0/_0$ ureum in bouillon 6—7 $^0/_0$ are changed into ammoniumcarbonate.

In culture-media containing the necessary anorganic salts together with ureum, a good growth is obtained with the following compounds after infection: pepton, asparagine, glucose, cane-sugar, maltose, citrates, lactates, tartrates, and salts of volatile fatty acids (except salts of formic acid).

§ 4. *Irisating cultureplates.*

The faculty in bacteria of splitting ureum can according to the method of BEIJERINCK by means of the yeast-water-gelatineplate 2 or 3 $^0/_0$ ureum, be proved in a very elegant way by the *Iris-phenomenon* formed on this culture-medium by those bacteria.

It is supposed that the ammoniumcarbonate getting free at the decomposition of ureum causes the phenomenon, in consequence of the precipitation of calciumcarbonate and -phosphate.

An explication of the origin of the irisphenomenon on the yeast-water-ureum-gelatineplate, has, however, its difficulties, the culture-plate being so complicated that it is not easy to get an exact idea of the process.

In the experiments with ureumbacteria on plates composed of water, 0.5 $^0/_0$ calcium salt of an organic acid, 1 $^0/_0$ ureum, 0.05 $^0/_0$ K_2HPO_4, 10 $^0/_0$ gelatine or 1.5 $^0/_0$ agar, the iris-phenomenon often produced itself.

The possibility of composing a simple culture-plate, if possible coagulated by agar, which produces the iris-phenomenon in a beautiful way, seemed not to be excluded, when the above facts were taken into consideration.

In this way corresponding phenomena on the yeast-water-ureum-gelatineplate and the irisating of more complicated culture-plates might be generally explained.

After some trials I succeeded in the following manner in composing a plate which entirely answers the requirements.

In pure water agar ± 0,5 $^0/_0$ calciummalate or -lactate and 0,05 $^0/_0$ ammoniumcitrate are dissolved; the melted agar is cooled down to the still just liquid state, after which a K_2HPO_4 solution is

added, till a slight opalizing is observed; now the culture-plate is formed of this material.

This culture-plate is, if made with care, almost clear. The calcium-phosphate that has been formed remains dissolved with the ammonium-citrate. A drop of ammoniumcarbonatesolution on this medium causes the irisphenomenon, while after some moments produces itself a precipitate round the drop.

This phenomenon shows itself in quite the same way, if, instead of agar, gelatine is taken.

The irisating field and the precipitate are microscopically and chemically identical to those which are produced on the yeast-water-ureum-gelatineplate.

If the culture-medium contains no phosphate, ammoniumcarbonate put on it gives a very slight field of $CaCO_3$; a drop of ammonia produces no irisating field at all.

If, however, only calciumphosphate, dissolved in ammonium-citrate, is present as the only calciumcompound, ammoniumcarbonate and also ammonia on such a plate cause an extremely fine irisating field.

If ± 2°/₀ ureum is added to this plate ureum-splitting microbes cause thereon the "*irisphenomenon*".

From these experiments it appears that the calciumphosphate-precipitation has to be considered as the real cause of the irisating of the culture-medium, whilst the calcium-carbonate formed at the same time plays a subordinate part.

Accordingly the irisating of culture-plates by certain bacteria growing on them and the *irisphenomenon* of BEIJERINCK have to be regarded as a consequence of the precipitation of calciumphosphate in the first and of calciumcarbonate in the second place.

§ 6. *Results obtained.*

1. Decomposition of ureum, in the absence of albumen, may take place by different microbes, if some suitable carbon-source is present.

2. In cultures in which ureum-splitting takes place, ± 98°/₀ of the total energy is developed by the decomposition of the ureum.

3. Cultures with calciumsalts of organic acids as a carbon-source, are extremely fit for getting weak splitting ureumbacteria. The *bacillus erythrogenes* occurring herein has been described more in detail.

4. Cultures with ammoniumsalts of organic acids or sugars as

a carbon-source, rapidly lead to the accumulation of strong ureum-splitting bacteria forming spores and the *urobacillus jakschii* forming no spores.

5. The irisating of culture-plates and the "irisphenomenon" on the yeast-water-gelatineplate are the consequence of the precipitation of calciumphosphate, whilst calciumcarbonate formed at the same time plays a subordinate part in it.

At the end of this investigation I beg to express my sincere thanks to Professor M. W. BEIJERINCK for advising and supporting me in these experiments wherever and whenever he could.

Physics. — "*Statistical Theory of Capillarity.*" By Dr. L. S. ORNSTEIN. (Communicated by Prof. H. A. LORENTZ).

(Communicated in the meeting of December 24 1908).

In a paper [1]) published in 1893 VAN DER WAALS has developed a theory of capillarity, which leads to results agreeing sufficiently with observation, as has been shown by the experiments of Dr. E. O. DE VRIES.

The methods used in the above mentioned paper have been reproduced with only a slight change in the lectures of VAN DER WAALS recently published by Prof. PH. KOHNSTAMM.

Both in the paper and in the treatise the hypothesis [2]) is introduced, that the entropy of an element of volume is a function only of the number of molecules it contains and of that of their collisions.

By the statistical method of GIBBS we can deduce the condition of equilibrium for the capillary layer without using a hypothesis of this kind and we can easily show that it must be true when certain conditions are fulfilled. This is the object of the present paper in which I shall also determine some quantities that play a part in the theory of capillary action.

§ 1. Let us suppose that n spherical molecules of diameter σ, perfectly rigid and elastic, are enclosed in a vertical cylinder of height Z, and of unit of horizontal section, closed at the top and the bottom by horizontal walls. Let the axis of z be drawn upward and let us further suppose that the molecules exert attractive forces on

[1]) J. D. v. D. WAALS, Thermodynamische theorie der capillariteit in de onderstelling van continue dichtheidsverandering. Verh. d. K. A. v. W. Deel I. 8. 1893.

[2]) Compare l. c, p. 16.