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Investigations on Flacherie.

BY

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Chapter I. Introduction.

Flacherie is a frequent disease of the silk-worm, causing great damage to sericulturists. The first observation was made by *Pasteur* who regarded this malady as being caused by three species of bacilli and a micrococcus. These microorganisms penetrated into the tissues of the larva and even into the eggs. *Cuboni*, however, was of the opinion that the pathogenic bacterium of this disease was a micrococcus which produced black spots on mulberry-leaves. Recently *Macchiati* [1] made investigations on this disease and concluded that the malady was caused by a streptococcus quite different from that found on mulberry-leaves which was a diplococcus. He recognized this streptococcus as the *Streptococcus bombycis*. He found besides this a bacillus in the digestive canal of the diseased larva, but he thought this bacillus could not cause the malady, its action consisting merely in the acceleration of the malady.

According to *Macchiati* the streptococcus and the bacillus have the following properties.

Streptococcus bombycis Macchiati.

The cell is round or oval, the diameter being 1.25—1,5 μ. It appears never isolated, but two, five or more unite in chains. It is aërobic; the propagation on potato is very quick and the colony thereon is greenish-yellow and has a metallic lustre. Colony on gelatine is light yellow, and gelatine is completely liquefied.

[1] Contribuzione alla Biologia dei Batteri bei Bachi affecti da flaccidezza. Le stazioni sperimentali Agrarie Italiane. Vol. XX. Part. II.

Bacillus bombycis Macchiati.

This bacillus exists not only in the larva, but also in the coccoon, crysalis and imago of silk-worm. The length is 1—3μ or often more; the ends of the rod are round, and two or more unite forming a long thread. The cell-membrane consists of cellulose, as it is coloured blue by iodine and sulphuric acid. It is motile and produces spores usually in the middle part of the rod. Colony on potato is yellowish-brown and elevated, which turns afterwards light brown.

Colony on gelatine is light yellow, and has a milky appearance, and gelatine is quickly liquefied. In gelatine stab-culture gelatine is completely liquefied, and flake-like precipitates are produced.

After the investigations of *Macchiati*, *Krassilschtschik*[1] in Pasteur's Institute made some investigations on this malady, and found that it is caused by a micrococcus quite different from that of *Macchiati*. This micrococcus has the following properties.

Streptococcus Pastorianus Krassilschtschik.

The cell is round, and has a diameter of 1—1.1μ. It occurs in the form of diplococcus. Colony on gelatine is round and gray, and gelatine is not liquefied. On gelatine stab-culture colony grows in nail-like form without liquefying it.

Macchiati[2] assumed that the micrococcus found by *Krassilschtschik* was the same as his streptococcus, disregarding the great difference of the properties between his and *Krassilschtschik's* microbe.

Macchiati[3] proposed to examine with a microscope the moth to be used for laying the eggs and to wash the eggs with sublimate solution, since the streptococcus exists usually in the moth and eggs. He said that very good results were obtained in various places by practising his proposal.

[1] Comptes rendus 1896. II. P. 427.
[2] Societa botanica italiana 1896.
[3] „ „ „ „

The microbes of flacherie were sometimes used for killing insects. In 1890 *Hoffmann*[1] used various species of microorganisms to kill *Laparis monacha*, injurious insect of forests, and found that *Botrytis* and the microbe of flacherie could become parasitic on this insect and kill it.

Tangl,[2] however, denied *Hoffmann's* report, since he did not believe *Bacillus bombycis* was the true pathogenic microbe of flacherie, and he said that bacteria could not be used for killing *Laparis*, as there was not known any microbes that were parasitic on that insect.

Tubeuf[3] described a certain bacterium which became parasitic on insects of pine-trees and also caused flacherie. The infected insect lost appetite and died finally. The contents of the intestines were brown, and numerous bacilli were found in it especially in the fore-intestines. He gave this bacillus the name of *Bacillus monachae*. Its width is 0.5μ and length 1μ. It was present also in the blood of the dead larva. This disease broke out more frequently, when the climate was cold and moist. *Tubeuf*, henceforth, explained the reason, that the disease was produced, because when it was cold and moist, the food was not digested well and remained long in the intestines, thus offering an opportunity for the growth of the bacillus.

In 1901 the Austrian Agricultural Experimental Station[4] reported that flacherie could not be infected to healthy silk-worms, neither by giving together with mulberry-leaves the intestinal juice of the diseased larva, nor the pure-culture of the microbe.

Omori[5] in Japan made investigations on this disease, and found that it was caused by four kinds of special micrococci and two kinds of special bacilli, and as the symptoms of the disease caused respectively by these microbes were different, he distinguished three kinds of flacherie.

[1] Central-Blatt für Bakteriologie XI, P. 341.
[2] „ „ „ XVI. P. 660.
[3] „ „ „ XII. P. 269.
[4] Oesterreich. Versuchsstationen IV. Part 3.
[5] Nihon Sanbyoron.

Chapter II,

Description of the Bacteria found in the Diseased Silk-worm.

The diseased larva lose appetite, vomit a viscous fluid and suffer from diarrhea or excrete a viscous fluid in most cases. When they are dead, the third and fourth segments become somewhat elongated and the whole body softenes. But there rarely occurs a case in which the dead body shrinks and becomes rather hard. The dead worm turns black usually very soon.[1] Sometimes while the diseased larvae are still living, the third and fourth segments are coloured black. In the digestive canal there are found usually a viscous fluid containing very little fragments of mulberry-leaves, but sometimes the digestive canal is filled with the fragments of mulberry leaves compressed to a hard mass. The color of mulberry-leaves therein is usually brown, while that in healthy animals is green. But rarely the mulberry-leaves in the intestines retain the original green color. While the reaction of the intestinal juice of the healthy larva is strongly alkaline, that of the diseased is in most cases neutral or only very faintly alkaline.[2] The fluid excreted shows often an acid reaction.

In the intestinal juice there are found a great number of bacteria. That abundantly found in many cases is a micrococcus. In some cases only micrococci are found, but in many cases there exist along with them large and short bacilli. There is sometimes a case in which short motile bacilli are seen, but it is very rarely observed, except in silk-worms reared in summer, that the large bacilli alone occur. Although these organisms exist abundantly in the intestinal juice of the diseased larvae, they can not easily

[1] According to *Dewitz* (Arch. für Anatomie und Physiologie 1902. P. 328) the turning black of the insect larva is due to oxydases. The fact that the diseased larvae turn usually very quickly black after death, or show black spots while still living, is probably due to the increased production of oxydizing enzyms accelerated by the insufficiency of nutrition as in the case of the vegetable cell which was proved by *Woods* (Centralb. für Bakteriologie II. Vol. 5. No. 22), or by the poisoning by nitrite.

[2] The digestive enzyms of *Lepidoptera* are active only in an alkaline solution, and lose their action in an acid solution. *S. Sawamura*. This Bulletin vol. IV. No. 5.

be detected in the tissues and blood of the diseased. It is clear from this fact that flacherie is caused by the propagation of the microbes in the intestinal juice.

By investigating silk-worms reared in spring, summer and autumn, the writer found that the micrococci in the intestinal juice are not of a single species but of many. But as their form is the same they can not be distinguished only by microscopical examination. The most remarkable difference is the color of the colonies on solid media. As in plate-cultures prepared from the intestinal juice of the diseased larvae, colonies of various colors made their appearance, it is beyond question that there exist in the intestinal juice of the diseased not only a single species but many species of micrococci (Fig. I and II). These microbes are present not only in the diseased larvae, but also in the healthy ones, although the number is small. Their presence in the latter case can be detected with a microscope or more easily by preparing plate-cultures.

To know whether the microbes are present in the interior of the eggs of silk-worm, they were washed with 0.1% sublimate solution and then with sterilized water, and crushed in bouillon, in which a micrococcus and a large bacillus propagated after few days. These experiments were repeated many times always with the same results. By examining the properties of the microbes, the large bacillus was found to be *Bacillus megatherium De Bary* and the micrococcus a Sarcina, the properties of which will be described later on. The presence of microbes in the interior of the eggs of insects other than silk-worm was observed by *Blockmann, Korschelt, and Zaccharis.*[1]

The properties of the microbes found in the intestinal juice of the diseased larvae and in the eggs are as follows ;—

The Large Bacillus.

Form: The cell, when cultured in bouillon for 24 hours, is 0.8μ. wide and 3—5μ long. In the intestinal juice it is larger. The extremities of

[1] Central-Blatt für Bakteriologie II. p. 546 and XI. p. 234.

the rod are round; and flagella grow on all sides, and are stained by *Löffler's* method. It exists isolated usually in the intestinal juice, but in nutritive fluid two or more are united.

Spore-formation: Spores are easily formed usually in the middle part of the rod.

Motility: It shows a slow oscillating motion.

Gram's method: Positive.

Oxygen: The growth is better in presence of air.

Bouillon: Propagation is good, and a cloud-like precipitate is formed and a feeble ring on the wall of the tube.

Gelatine streak: Gelatine is quickly liquefied along the inoculated line.

Agar plate: Colony having curl-like appearance, irregularly extending from a light brown point formed in the centre.

Agar streak: A dirty white colony is formed on the whole surface.

Agar stab-culture: Colony is formed straightly along the inoculated line to the bottom, and it propagates on the surface quickly to the wall of the tube.

Potato: An elevated gray colony within 2 days of inoculation at 20°c.

Milk: Milk is coagulated but not with an acid reaction.

Reduction: Nitrate is reduced to nitrite as shown by the iodine-starch and *Griess'* reaction.

Gas-Production: Gas is not evolved by cultivating in a nutritive solution containing glucose.

H_2S: A trace of H^2S is formed by cultivating it in bouillon.

Acid-production: By cultivating for 3 days at room-temperature in a nutritive solution containing 5% of glucose (with Ca CO_3), there was produced 0.153% of acid, calculated from the dissolved CaO, as lactic acid.

By these properties this bacillus is proved to be *Bacillus megatherium De Bary*.

The Short Bacillus.

Form: The cell cultured in bouillon for 24 hours is 0.6μ wide and 1.0—1.5μ long. It is isolated both in the intestinal juice and in bouillon,

and very rarely two are united. The extremities of the rod are round. Flagella are colored by *Löffler's* method, and some have them in one end, while the other on all sides.

Spore-formation: Spores are not formed.

Mobility. It moves actively.

Gram's method: It is not colored by *Gram's* method. But some absorb colors especially well in the ends of the cell.

Bouillon: Bouillon becomes turbid and viscous. The precipitate formed can be easily distributed by shaking.

Gelatine plate: A white round colony is formd without liquefying gelatine.

Gelatine stab-culture: Colony is formed straightly along the inoculated line to the bottom, and on the surface a white colony is formed which extends to the wall of the tube. The centre of the colony assumes a light yellow color after some days.

Agar streak: A moist, bright, white colony is formed.

Potato: An elevated yellow colony is formed.

Milk: Milk is coagulated, acid reaction being produced.

Gas-production: Gas is evolved by cultivating it in a nutritive solution containing glucose.

Reduction: It reduces nitrate to nitrite.

Indol reaction: A faint red color is produced in pepton-water culture (for 24 hours at 25°C), when it is warmed with addition of H_2SO_4 or HCl.

Acid: It produces acids in a solution containiug glucose.

By these properties this bacillus is proved to be the coli-bacillus.

The Micrococcus I.

Form: The cell cultured in bouillon for 24 hours has a diameter of about 0.8μ, and appears usually in the form of diplococcus.

Gram's Method: Positive.

Oxygen: Growth is better in presence of air.

Bouillon: A little white precipitate was formed, when cultured for 2 days at 23°C. No scum was formed, although kept for more than 2 days.

Gelatine plate: A yellow, round, sharply defined, moist, bright, homo-

genous and elevated colony, that does not liquefy gelatine, is formed on the surface. By weak magnification the appearance is the same. Deep colony is a white point.

Gelatine streak: Colony is homogenous, and at first white but afterwards turns yellowish brown: It does not liquefy gelatine.

Gelatine stab-culture: Colony is formed straightly along the inoculated line to the bottom.

Agar streak: Colony is elevated, homogenous, moist, and at first white but afterwards assumes a faint brown.

Potato: A white, homogenous colony is produced along the inoculated line in 6 days at 23°C.

Milk: Milk is coagulated, acid reaction being produced.

Gas-production: Gas is not evolved.

H_2S: H_2S is not formed.

Reduction: Nitrate is reduced to nitrite.

Acid: Acid are produced when cultivated in a nutritive glucose bouillon.

The Micrococcus II.

Form: The cell cultivated in bouillon for 24 hours is about 0.8μ in diameter. It occurs usually in the form of diplococcus, but sometimes four are united.

Gram's method: Positive.

Oxygen: Aërobic.

Bouillon: At 15°C on the fourth day of inoculation it becomes turbid, and on the sixth day a precipitate is formed, the supernatant fluid remaining clear. It is the same after 20 day's culture.

Gelatine plate: Surface colony is round, convex, sharply defined, homogenous, moist, bright, white and has a porcelain lustre. Gelatine is not liquefied. Weakly magnified appearance is the same as the above. Deep colony is a white point.

Gelatine stab-culture: Colony is formed straightly to the bottom along the inoculated line.

Gelatine streak: A white, moist, homogeneous, elevated colony is formed along the inoculated line, without liquefying the media.

Agar streak: A white, moist, homogeneous, elevated colony is formed which extends very soon the whole surface.

Potato: A white, homogeneous, moist, elevated colony is formed on the fourth day of inoculation at 30°C.

Milk: Milk is coagulated, acid reaction being produced.

Gas-production: Gas is not evolved.

H_2S: H_2S is not formed.

Reduction: Nitrate is reduced to nitrite.

Acid: Acids produced by cultivating in a nutritive glucose solution (with Ca CO_3) for 6 days at 36°C. was found to be 0.12% calculated from the dissolved Ca O as lactic acid.

The Micrococcus III.

Form: The cell cultured in bouillon for 24 hours is about 1 μ in diameter. Usually two are united but sometimes four.

Gram's method: Positive.

Oxygen: Aërobic.

Bouillon: It becomes turbid by two day's cultivation at 23°C. After 20 days a feeble scum is formed, and a yellow precipitate on the bottom, the supernatant fluid remaining still turbid.

Gelatine plate: Surface colony is yellow, round, convex, sharply defined, moist and bright. By weak magnifying power the appearance is the same, granular consistence being visible. Gelatine is liquefied. Deep colony is a white point.

Gelatine stab-culture: Colony is formed straightly to the bottom along the inoculated line, liquefying it in the shape of a nail.

Gelatine streak: A sulphur-yellow colony is formed along the inoculated line, liquefying it completely after a few days.

Agar streak: An elevated moist homogeneous colony is formed, the color of which is white at first, but turns sulphur-yellow after a few days.

Potato: A very elevated, moist, bright, homogeneous colony is formed

along the inoculated line. It is yellow at first, but turns brown after a few days.

Milk: Milk is coagulated, acid reaction being produced.

Gas-production: Gas is not evolved.

H_2S: H_2S is not formed.

Reduction: Nitrate is reduced to nitrite.

Acid: Acids were produced by cultivating in pepton-water containing glucose.

In the intestinal juice other micrococci and bacilli are of course present, although their number is commonly less than the above described. But as the colonies formed by those micrococci which are chromogenous, are at first white and assume the proper tint after many days of culture, mistakes are possible by not giving time enough

The Micrococcus present in the Eggs.

Form. The cell cultivated in bouillon for 24 hours is 1 μ in diameter. It occurs always in packet-form in nutritive fluids. But in the intestinal juice of the larvæ it occurs in the form of diplococcus.

Gram's method: Positive.

Oxygen: Aërobic.

Bouillon: Bouillon becomes turbid little on the seventh day of inoculation, and a light yellow precipitate is formed after 20 day's culture.

Gelatine plate: Surface colony is yellow-moist, bright, elevated, round and sharply defined. By weak magnification it is granular. Deep colony is a yellow point. Gelatine is liquefied.

Glatine streak: A yellow, elevated colony is formed along the inoculated line, gelatine being quickly liquefied.

Gelatine stab-culture: Colonies are formed discontinously along the inoculated line. Gelatine is liquefied at first in the shape of a nail, but afterwards in the shape of a cylinder.

Agar plate: Surface colony is yellow, moist, bright, non-tenacious, elevated, round, sharply defined, and has a point on the centre. By weak magnification granular consistence is visible. Deep colony is a white point.

Agar streak: An elevated, especially in the central line, moist, homogeneous colony, the color of which is yellow shadowed with black, is formed.

Potato: A yellow, moist, homogeneous colony is formed along the inoculated line on sixth day at 23°C.

Milk: Milk is coagulated with much production of acid.

Gas-production: Gas is not produced.

H_2S: H_2S is not formed.

Reduction: Nitrate is reduced to intrite.

Acid: Acids produced in 14 day's culture in glucose-bouillon at 20°C. was 0,33% calculated as lactic acid.

Yellow pigment of the micrococcus is insoluble in water, alcohol or ether, but soluble in potash solution, which turns pale red by warming with addition of HCl.

By these properties this microbe is recognized as *Sarcina lutea Flügge*.

Chapter III.

The results of the experiments.

Since in the intestinal juice of the diseased larva, an abundant growth of bacteria takes place, it is certain that this malady is caused by these microorganisms. But as these bacteria make luxuriant growth only in the intestinal juice and never invade considerably the tissues or blood, the pathogenic action will perhaps be due to the production of a certain toxin. Hence some experiments were undertaken to test this suggestion by using a solution, containing toxin, prepared in the usual manner from the culture of the micrococci commonly found abundantly in the diseased larva.

Experiment I.

This experiment was performed in this College in October of 1901. At

this time it was rather cold and since flacherie happens more rarely in cold than in warm weather, the silk-worms used for the experiment were reared in a large box constructed to keep the larva at a somewhat elevated temperature. This box and other apparatus used in the experiment were sterilized with the vapors of formalin.

The material used for this was prepared from *Micrococcus II* cultured in bouillon for 9 days at 36°C. The filtrate was prepared from the above culture by filtering through *Chamberland's* filter; and as in some cases toxin is not secreted from the living bacteria cells, a part of the culture was heated to 65°–70°C. for 30 minutes to kill the bacteria-cells.

Oct. 29. 3 P.M. The original culture, the filtrate and the heated culture were given together with mulberry-leaves to the larvæ of the second day of the fourth age. The number of the larvæ used for each experiment was 20, and the quantity of the materials used was 1,5 cc. to 100 grs. of mulberry-leaves. The larvæ showed a very good appetite, and then they were treated as usual. The temperature in the box was 21°C.

Oct. 30. When they were examined in the morning, there were no diseased larvæ found. Hence the culture, the filtrate and the heated liquid were given again to the larvæ as before, and the temperature was raised to 25°C. and water was besprinckled in order to increase moisture, because high temperature and moisture are favorable to the development of flacherie. But as the arrangement to keep the temperature high was imperfect, it fell too low during the night.

Oct. 31. No symptom of the disease was observed in all the sections.

Nov. 1. All the larvæ, except one in the control experiment that had died, span healthy cocoons. An excreta of the larvæ fed with the culture of the micrococcus was put into bouillon, in which the micrococci made luxuriant growth after a few days, proving that micrococci had entered and passed the digestive canal of the larva.

Experiment II.

The negative result obtained in the former experiment might have been due to the low temperature. So this experiment was performed to repeat the former one using higher temperature. The culture used for this was also that of *Micrococcus II* cultured in bouillon for 3 days at 36°C. Filtration and heating were performed as in the former experiment.

Nov. 8. In the afternoon the materials were given twice respectively to 20 of the larvæ of the fourth day of the fourth age in the same manner as in the former. The intestinal juice of the diseased larvæ was also given to 10 larvæ. At 4 P.M. they were put in a thermostat and kept at 27°C. In the thermostat the ventilation was rather poor and the moisture content high, so that moulds grew on the excreta. The larvæ soon got into the stage of ecdycis, and on 10th they ended ecdycis. From this day on death took place.

Nov. 11. The silk-worms were transfered to a room of 21°C.

The number of the dead larvæ will be seen from the following table.

Date.	Control.	The culture.	The filtrate.	The heated culture.	The intestinal juice.
Nov. 10	1	4	1	0	7
11	0	0	0	0	0
12	0	1	0	1	0
13	0	1	0	0	2
14	0	0	1	0	0
15	0	0	0	0	1
16	0	0	0	0	0
17	1	0	2	0	0
18	0	0	0	0	0
19	0	0	0	0	0
20	0	0	0	0	0
21	1	4	0	0	0
Total	3	10	4	1	10

The remainder formed coccoons.

As soon as the larvæ died, their intestinal juice was examined with a

microscope, and according to the microbes present in the juice and also other symptoms, the disease of the dead larvæ was grouped as follows:—

	Control.	Culture.	Filtrate.	Heated culture.	Intestinal juice.
Flacherie[1] I	0	7	1	0	7
„ II	1	0	0	0	1
Grasserie	0	1	1	0	2
Pebrine	2	2	3	1	0
Total	3	10	5	1	10
Flacherie in % of total larvæ	5	35	5	—	8

Contrary to the former experiment many flacherie-patients were produced in this. It can, therefore, be concluded:—

(I), that flacherie takes place when temperature and moisture are high and ventilation is insufficient, in short, when the conditions are injurious to the health of the silk-worms;

(II) that pathogenic action is not due to the production of toxin.

Experiment III.

This experiment was performed to confirm once more the result of the former ones. The cultures used in this experiment were prepared from *Micrococcus II* cultivated in bouillon for 10 days at 36°C. and from *Micrococcus I* cultivated in bouillon for 34 days at 36°C. The filtrate was however prepared only from the former.

Nov. 14. At noon the cultures and other materials were given to the larvæ on the second day of the fifth age, taking 20 larvæ for each experiment. The temperature of the room was 15°C. and moisture 54. They were kept to the 18th, no diseased one being observed. They were therefore placed in a thermostat and kept at 27°C. and on the 22nd they

[1] Flacherie I denotes that in which micrococci were abundant, and flacherie II where bacilli were abundant.

were again transfered to the former room, and on the 26th they formed coccoons.

The number of the dead larvæ during the experiment was as follows:—

Date.	Control.	Culture of *Micrococcus I.*	Culture of *Micrococcus II.*	The filtrate.
Nov. 19	3	1	2	1
20	1	2	1	3
21	2	2	0	2
22	0	1	0	0
23	0	0	0	0
24	0	0	0	0
25	1	4	0	1
Total	7	10	3	7

The disease was grouped as follows:—

	Control.	*Micrococcus I.*	*Micrococcus II.*	Filtrate.
Flacherie I.....	3	5	2	7
„ II.....	2	4	1	0
Grasserie	0	1	0	0
Pebrine	2	0	0	0
Total	7	10	3	7
Flacherie in % of the total larvæ	25	45	15	35

It will be seen from these tables that flacherie was more in the larvæ that were not infected artificially, than in those that received the bacteria. From this fact it can be learned that the bacteria, that cause flacherie, are already present in the vicinity of the larvæ and even in their intestines, waiting for an opportunity for development. Since many patients appeared among the larvæ fed with the filtrate, flacherie would seem to be caused by

some toxins. But this can not be sure, because in the intestinal juice of the diseased larvæ many micrococci were present which certainly had caused the malady.

The results obtained in this and other experiments disprove the infectiousness of flacherie, which is against the belief held by the sericulturists of the present day.

Experiments IV.

This experiment was performed to investigate once more the pathogeny of the micrococci.

1902. May 8. *Micrococcus II* cultured in the decoction of mulberry-leaves for 7 days at 36°C. were given four times to 100 larvæ (*Aohiki* variety) of the first day of the first age. After this they were kept in the usual manner till the fourth age, without observing any symptoms of flacherie.

The number of the larvæ examined on the first day of the fifth age were as follows:—

	Healthy	Dead	Lost
Control	89	6	5
Inoculated	91	3	6

The average temperature and moisture during the experiment were as follows:—

	Temperature	Moisture
First age	19,0°C.	68,4
Second „	21,5	71,4
Third „	22,0	75,3
Fourth „	20,0	78,1

Experiment V.

Since flacherie occurs usually more in old larvæ than in young ones, the negative result obtained in the former experiments might be due to the fact

that the bacteria were fed to young larvæ. Therefore this experiment was repeated, using old larvæ.

The bacteria used for this experiment were *Micrococcus II* isolated this year from a diseased larva and the sarcina[1] isolated from the eggs of silkworm. They were inoculated with the following materials.

I. The micrococci, cultured on agar, suspended in water.

II. The filtrate obtained from the decoction of mulberry-leaves cultured for a week at 36°C.

III. The above culture heated for 30 minutes to 65°C.[2]

IV. The same to which formalin was added in the proportion of 1 drop to 10 cc. of the culture.[3]

May 22. 3 P.M. The materials above described were given together with mulberry-leaves[4] each to 100 larva (*Akahiki* variety) on the first day of the third age. They were kept in the usual manner till the fifth age without observing any symptoms of the desease.

The average temperature and moisture during the experiment were as follow:—

Date.	Temperature.	Moisture.
May 22	22,5°C.	70,0
23	22,0	78,0
24	22,1	80,0
25	21,1	75,0
26	22,0	82,0
27	22,0	69,0
28	18,5	74,7
29	22,2	78,7

[1] *Sarcina lutea Flügge*.

[2] Sterilized.

[3] Sterilized.

[4] 1,5 cc. of the materials to 100 grs. of mulberry-leaves.

Experiment VI.

As the result of the former experiments were all negative, it seemed doubtful that the bacteria used in these experiments were not the pathogenic ones. This experiment was therefore performed to observe the infective power of the intestinal juice of a diseased larva.

May 28. 3 P.M. The intestinal juice, obtained respectively from a dead larva whose body was softend and elongated, and from that whose body was contructed, were fed four times together with mulberry-leaves each to 10 larvæ (*Akahiki* variety) of the second day of the fifth age, In the intestinal juice there were of course bacilli and micrococci in great number. They were then fed in the usual manner for a week without observing any symptoms of the disease. The average temperature during the experiment was as follows:—

Date.	Temperature.
May 28	17,0°C.
29	18,0
30	20,0
31	19,5
June 1	21,5
2	19,8
3	20,9

From these experimental results it is clear that silk-worms do not become ill from flacherie when the surrounding conditions are favorable to their health, and they have resistance-power. These results agree with that reported by the Austrian Agricultural Experimental Station.

Experiment VII.

Since the negative results obtained in the former experiments might have been due to the insufficiency of the number of the bacteria, a further

experiment was made by injecting various bacteria directly into the intestines through the anus by means of a syringe, the point of which was carefully rounded off. The bacteria cultured on agar were suspended in water and 0,05 cc. were injected. The species of the bacteria used were as follows:—

Micrococcus I.
„ *II.*
„ *III.*
Coli-bacillus (isolated from the diceased larva).
Bacillus mesentericus vulgatus Flügge.
„ „ *fuscus* „
Bacillus subtilis Cohn.

June 7. 2 P.M. The above materials were injected into the larvæ (*Aohiki*-variety) on the second day of the fifth age. After the silk-worms had recovered the normal state which took about five hours, 10 lively larvæ were selected from each section, and at the same time 100 larvæ were kept as control, among which no disease appeared during the experiment.

According to a previous experiment it was known that flacherie is produced after about three days even by injecting pure water into the intestines through the anus, when the temperature is high, but when bacteria are injected the malady is produced more quickly. A slow development of the disease at a high temperature therefore would give naturally no decisive result. The results of the injection must be observed within 3 or 4 days. The results after three days were as follows:—

1. *Distilled Water.*

The larvæ injected behaved very lively and showed a very good appetite. On the third day two of them died; in their intestinal juice many micrococci were found.

2. *Micrococcus I.*

The silk-worn s lost appetite. On the afternoon of the second day four

of them died of flacherie; in the intestinal juice micrococci and *Bac. megatherium* were found in large number. On the same night one died, in whose intestinal juice only the micrococci were found. On the night of the third day two more died, in which also only the micrococci were found.

3. *Micrococcus II.*

The larvæ lost appetite. On the afternoon of the second day one died of flacherie, in which much of *Bac. megatherium* and little of micrococci were found. On the second night three died, in which a great number of micrococci was found.

4. *Micrococcus III.*

The larvæ lost appetite. On that night two died of flacherie, in one of which the micrococci prevalent, while in the other *Bac. megatherium* exceeded the number of micrococci. On the second day five died of flacherie, in which a great number of micrococci was found.

5. *Coli-bacillus.*

The larvæ lost appetite completely. On the afternoon of the second day six died of flacherie in which coli-bacilli were found in great number. On the second night three died, and on the afternoon of third day one died. In the former only coli-bacilli, while in the latter *Bac. megatherium* was found.

6. *Bacillus mesentericus vulgatus Flügge.*

The larvæ lost appetite completely. On the afternoon of the second day two died, in one of which *Bac. mes. vulgatus* prevailed, while in the other micrococci were more abundant. On the second night seven died, in six of which *Bac. mes. vulgatus*, but in one only micrococci were observed.

7. *Bacillus mesentericus fuscus Flügge.*

The larvæ lost appetite. On the second night seven died of flacherie, in four of which only *Bac. mes. fuscus*, but in three this microbe together with micrococci were found. On the afternoon of the third day one died, in which *Bac. mes. fuscus* alone was observed.

8. *Bacillus subtilis Cohn.*

The larvæ did not lose appetite so completely as the others. In the first night one died of flacherie, in which much *Bac. subtilis* was found. On the forenoon of the third day four, and on the afternoon one died of flacherie; in the former *Bac. subtilis* prevailed, while in the latter micrococci.

The results of the experiments may be summerized in the following table.

	First day.	Second day.	Third day.	in % of the larvæ taken for the test.
Control	0	0	0	0
Water	0	0	2	20
Micrococcus I	0	5	2	70
„ *II*	0	4	0	40
„ *III*	2	5	0	70
Coli-bacillus	0	9	1	100
Bac. mes. vulgatus	0	9	0	90
„ „ *fuscus*	0	7	1	80
Bac. subtilis	1	0	5	60

From the results obtained in this experiment the following conclusions can be drawn:—

1. Many species of bacteria can propagate in the intestinal juice of the larvæ and cause flacherie.

2. Disorder in the digestive organ such as injection of water causes flacherie.
3. From the above facts it is clear that bacteria, that can cause flacherie, are present at all times in the intestinal canal of the larvæ waiting for an opportunity for development.
4. That flacherie caused by the injection of the bacteria is not due merely to the disorder in the digestive canal, is proved by the following facts.
   a. The bacteria injected into the intestines multiplied therein.
   b. When bacteria were injected, flacherie was produced more quickly and frequently than when water is injected.

Experiment VIII.

This experiment was performed to test once more for the production of toxin by injection. The materials used for the experiment were *Micrococcus III* cultured in a decoction of mulberry-leaves in absence of air for 7 days at 36°C.

It was filtered through *Chamberlana's* filter and a part of it was neutralized with $Na_2 CO_3$.

June 9. 2 P.M. 0,05 cc of the original and the neutralized filtrates were injected into the intestines of the larvæ (*Aohiki* variety) on the fourth day of the fifth age. The results were as follows:—

1. *The Original Filtrate.*

Eleven larvæ which received this material remained inactive for two hours. One of them was killed and the intestinal juice was examined, in which *Bac. megatherium* was found in large number. On the next morning eight larvæ died, in two of which micrococci abounded, but little of *Bac. megatherium* was present; and in three others *Bac. megatherium* abounded, while micrococci were few; while in three others only *Bac. megatherium* was found. On the 11th two died, in which *Bac. megatherium* abounded, but few micrococci were found.

2. *The Neutralized Filtrate.*

On the forenoon of the 10th nine out of twelve larvæ used for the operation died, in which *Bac. megatherium* abounded. On the 11th three died, in which both *Bac. megatherium* and micrococci were found.

Experiment IX.

Since some bacteria produce a powerful toxin only when they are mixedly infected, all the bacteria isolated from the diseased larvæ were cultured together in nutritive glucose solution for 24 hours at 36°C. A culture of coli-bacillus also served.

June 10. 11 P.M. 0,1 cc. of the original and neutralized filtrate of the above cultures were injected into the larvæ (*Aohiki* variety) on the fifth day of the fifth age.

The number of the dead was as follows:—

	Number of the larvæ tested.	First day.	Second day.	Third day.	Fourth day.	% of the dead.
Water	10	0	0	2	0	20
Filtrate from coli-bacillus	10	0	6	4	0	100
The same neutralized.	10	0	4	4	2	100
Filtrate from the mixed culture	10	0	6	4	0	100
The same neutralized.	10	0	5	2	2	90

According to the kinds of the bacteria found in the intestinal juice they may be grouped as follows:—

	Much *Bac. megatherium.*	*Bac. megatherium* + micrococci.	*Bac. megatherium* + coli-bacillus.	*Bac. megatherium* + coli-bacillus + micrococci.
Water	0	2	0	0
Filtrate from coli-bacillus.	1	7	0	2
The same neutralized ...	0	3	2	5
Filtrate from the mixed culture	4	3	3	0
The same neutralized ...	4	1	0	4

As many died of flacherie in this experiment, it might be supposed that the malady was caused by toxins, but that is very improbable, since water alone might have produced the same result.[1]

Moreover flacherie is caused by various kinds of bacteria as was shown in the previous experiments. This makes it very improbable that a specific toxin is the cause of the malady.

Experiment X.

From the results obtained in the previous experiments there is no doubt that flacherie is not caused by a special toxin. But since the malady is caused by the multiplication of bacteria in the intestinal juice, the cause of the disease must be due to some action of bacteria and since many kinds of bacteria can produce this disease, the injurious action must be one common to all these bacteria.

The vital action common to all of them and suspicious of injury to the silk-worm is the formation of acid, because the digestive enzyms of silk-worm are active only in an alkaline solution. The micrococci found in the diseased larvæ and that in the eggs as well as *Bac. megatherium, Bac. coli, Bac. subtilis, Bac. mes. vulgatus and fuscus*, all produce acids in a solution containing carbohydrates, which were comfirmed by direct experiments. Moreover, since the reaction of the intestinal juice is neutral or faintly alkaline, and the fluid excreted in flacherie is sometimes quite acid, there must exist some relation between flacherie and the formation of acids by the bacteria. Hence the effect of injection of acids was studied.

0,1 cc. of distilled water, 3% normal sodium carbonate solution, 2% acetic acid, 2% lactic acid and 2% butyric acid were respectively injected, as in the former experiments, into the intestinal canal of the larvæ of the fifth age. By this operation some vomited fluid, especially many of those injected with water and sodium carbonate solution. All the larvæ seemed somewhat inactive, but those injected with water and sodium carbonate solution showed very good appetite after a few hours.[2]

[1] Compare also the above experiment, p. 420.

[2] The larvæ injected with distilled water did not die within 24 hours.

Those injected with the acids died with vomition and diarrhea after about 10 hours, the dead bodies softened, the third and fourth segments being elongated; in short showing the close resemblance to those died of flacherie.

Those injected with 0,1 cc. of 10% lactic acid died instantly without vomition or diarrhea, the bodies contracting and becoming rather hard. But even in this case the intestinal juice of the dead did not show an acid reaction, but still was alkaline, what shows that the silk-worm even dies when the alkaline reaction of the intestinal juice is a little weakened. By this experimental results it may be explained, why the appearance of the dead bodies of the larvæ in one case is different from that in the other.

Vomition and diarrhea characteristic to flacherie is probably due to the fact, that as the intestinal juice is neutralized by the acids produced by the bacteria, the patient secretes more juice to restore the alkaline reaction on the one hand, while resorption is stopped on the other; hence the quantity of the fluid in the intestinal canal increases so much as to cause vomition and diarrhea.[1]

Experiment XI.

But the bacteria seem to produce a certain poison, although it is no toxin. It is a well known fact that the coli-bacillus reduces nitrate to nitrite. But the production of nitrite in the decoction of mulberry-leaves by *Bac. megatherium* and the micrococci were also proved by the writer.[2]

This experiment was performed therefore to observe the effect of nitrite on the silk-worm.

July 4. 9 A.M. 20 larvæ of the second day of the fifth stage were fed with mulberry-leaves moistened with a 10% solution of sodium nitrite for a day.[3] On the next morning a larva died.

[1] In higher animals also the secretion of the intestinal juice is much accelerated by presence of acids. *Bunge*. Physiol. Chemie.

[2] Mulberry-leaves contains often much nitrate.

[3] 1.5 cc of the solution to 100 grs. of the leaves. The larvæ did not eat the leaves as usual.

They were then fed with the normal leaves, but on the sixth day two died with vomition and diarrhea, but bacteria were not observed in the intestinal juice as in the case of flacherie.

By injecting 0,1 cc. of 1% solution of sodium nitrite in the usual manner, six out of seven larvæ used for the experiment died instantly, the bodies of which were softened and stretched. Those to which only 0.05 cc. were injected, were, for 10 hours after the operation, in a somnolent condition. Then they became again active, but on the third day five died; the dead bodies becoming softened. With the intestinal juice of the larvæ that died of flacherie, the usual nitrate reactions can sometimes distinctly be obtained. These facts make it clear that nitrite formed by bacteria is one of the injurious products that may contribute to the development of flacherie.

Experiment XII.

Since *Bac. megatherium* or *Bac, coli* are of general occurrence it is no wonder that they propagate also in the intestines of the silk-worms. But as to the micrococci it is different.

As a micrococcus and *Bac. megatherium* exist in the interior of some eggs, they might come from the eggs as *Pasteur* and *Macchiati* supposed. But flacherie is usually prevalent after the fourth stage.

Therefore it is very improbable that the micrococcus remains in the digestive canal for so long a time without developing the malady. Moreover the micrococcus found in the eggs was quite different from those usually found in the diseased larvæ.

1902 June 23.[1] Agar-plates were infected with small fragments of a mulberry-leaf.

The colonies formed after two days were as follows:—

The original plate: Colonies of a large bacillus (*Bac. megatherium* or *Bac. subtilis* ?)

The second dilution: Numerous colonies of the large bacillus and micrococci.

[1] It rained two days before.

The third dilution: Colonies of the large bacillus and white colonies of micrococcus.

June 24. The former experiment was repeated, as on the previous day there had been a heavy rain.

The results were as follows:—

The original plate: Colonies of the large bacilli and micrococci intermingled.

The second dilution: Colonies of the large bacilli and white and brown colonies of micrococcus.

June 27. The experiment was repeated with mulberry-leaves of *Harajuku* where silk-worms were never reared before.

The results were as follows:—

The original plate: Colonies of various bacteria covered the whole surface.

The second dilution: Yellow and gray colonies of micrococci besides those of other bacteria.

The third dilution: White and light brown colonies of micrococcus.

To decide whether the micrococci of mulberry-leaves are the same as those of flacherie, it was necessary to observe their action on the silk-worm.

July 7. 9 A.M. The micrococci isolated from mulberry-leaves and those of the diseased larvæ were inoculated in the usual manner into the larvæ of the fifth day of the fifth stage, and as they died, their intestinal juice was examined with a microscope.

The results were as follows:—

	Number of larvæ.	First day.	Second day.	Third day.	Total.	% of the dead.
Water	10	0	1	2	3	30
Micrococcus II of silk-worm	10	0	5	2	7	70
Bac. megatherium from silk-worm.	10	2	8	0	10	100
Micrococcus I from mulberry-leaves[1]	10	0	4	6	10	100
Micrococcus II from mulberry-leaves[1]	10	0	5	5	10	100
Control	36	0	0	0	0	—

[1] *Micrococcus I* from mulberry-leaves formed a white colony, while *Micrococcus II* a light brown colony on agar.

The dead larvæ were grouped according to the species of the bacteria found in the intestines as follows:—

	Micrococcus only.	*Bac. megatherium* and micrococci.	*Bac. megatherium* only.	*Bac. megatherium* and coli-bacillus.	Very few bacteria.
Water	1	1	1	0	0
Micrococcus II of silk-worm.	2	3	1	1	0
Bac. megatherium	0	0	8	0	2
Micrococcus I from mulberry-leaves	3	4	3	0	0
Micrococcus II from mulberry-leaves	9	1	0	0	0

From these results it follows that the micrococci present on the mulberry-leaves can cause flacherie in just the same manner as those from the diseased silk-worms. It is therefore very probable that the micrococci found in the intestinal juice are the same as those found on mulberry-leaves.

Experiment XIII.

1902 October. In order to observe whether the micrococci found in the diseased larvæ exist also on mulberry-leaves or not, micrococci were isolated from these leaves and their properties were examined.[1]

No. 1.

Form: The diameter of the cell cultivated in bouillon for 24 hours is 1 μ. Commonly two are united. The cells are colored by *Gram's* method.

Bouillon: At 15°–17°C. bouillon becomes turbid on the second day of inoculation, and on the seventh day a white ring is formed on the wall of the tube and a white precipitate is formed, the supernatant fluid becoming clear. After 20 days a feeble scum appears.

Gelatine plate: Surface colony is white, round, sharply defined, lipped,

[1] The leaves came partly from the College farm in *Komaba*, and partly from a garden in *Tobio* where no silk-worms were kept for 30 years.

moist and has porcelain-like lustre. A point of light brown color is in centre. By weak magnification the appearance is the same, showing a curled consistence. Deep colonies appear as white points.

Gelatine streak: Colony light brown, folded, a film is found along the inoculated line, gelatine being liquefied.

Gelatine stabculture: At 12°C. after 20 day's culture, colonies are formed along the inoculated line, liquefying gelatine.

Agar streak: White, homogeneous, moist, elevated, tenacious colony.

Potato: At 23°C. elevated white colonies are found which on the sixth day turn brown and show granular consistence.

Milk: At 30°C. milk is coagulated in 24 hours, acids being formed.

Oxygen: Growth is better in presence of air.

Gas: Gas is not evolved by cultivating in a nutritive solution containing glucose for 7 days at 15°C.

H^2S: H^2S is not observed in bouillon cultured for 24 hours.

Reduction: Nitrate is reduced to nitrite.

Acids: Azolithmin is turned red in pepton-water cultures containing 5% of glucose. This micrococcus is therefore probably *Micrococcus coronatus Flügge*.

No. 2.

Form: The diameter of the cell cultured in bouillon for 24 hours is 0.8 μ. Usually two are waited. The microbe is colored by *Gram's* method.

Bouillon: On the fifth day it becomes turbid, and on the seventh day a yellowish brown precipitate is formed. After 20 days a feeble brown scum appears.

Gelatine plate: It did not grow on gelatine within 13 days at room-temperature (winter).

Gelatine streak: Granules of white and light yellowish color are formed intermingled along the inoculated line. Their color changes afterwards respectively to yellow and deep brown. Gelatine is slowly liquefied.

Gelatine stabculture: Thread-like growth to the bottom and liquefaction in the form of a nail.

Agar plate: At 30°C. the surface colony is light brown moist, bright,

round, lipped, sharply defined, and the centre is somewhat elevated. By weak magnification its consistence seems to be homogeneous. Deep colonies appear as white points.

Agar streak: White, moist, granular, non-tenacious colony which assumes after a few days a yellow color.

Potato: On the second day a flat, dry, light brown, granular colony along the inoculated line.

Milk: It is coagulated showing alkaline reaction.

Oxygen: Aërobic.

Gas: Gas is not evolved.

H_2S: H_2S is not formed.

Indol reaction: Faint reaction.

Reduction: Nitrite is formed from nitrate.

Acids: Acids are formed in glucose solution.

This micrococcus is *Micrococcus bicolor, Zimmermann.*

No. 3.

Form: The cell cultured in bouillon for 24 hours has a diameter of 0.8 μ. Two are usually united, but sometimes four, isolated cells are rare. It is colored by *Gram's* method.

Bouillon: At 23°C. on the second day a little white precipitate is formed, the supernatant fluid becoming clear. It is the same after 20 days.

Gelatine plate: Surface colony is dirty white, round, convex, with a white ring. By weak magnification, the centre seems deeply colored and it becomes lighter towards the margin. Gelatine is slowly liquefied.

Gelatine streak: A light brown, homogeneous colony is formed, gelatine being liquefied.

Gelatine stabculture: Thread-like growth to the bottom, liquefying gelatine along the inoculated line.

Agar streak: Moist, homogeneous, tenacious colony which is white at first, but becomes light reddish brown afterwards.

Potato: It did not grow on potato in a week at 30°C.

Milk: Milk is coagulated, acids being formed.

Oxygen: Aërobic.

Gas: Gas is not formed.

H_2S: H_2S is formed.

Reduction: Nitrate is reduced to nitrite.

Acid: Acid is formed in glucose solution.

No. 4.

Form: The cell cultivated in bouillon for 24 hours has a diameter of 1 μ. Two are usually united. It is colored by *Gram's* method.

Bouillon: At 23°C. on the second day a white precipitate is formed. Scum is not formed by 20 days' culture.

Gelatine plate: Surface colony is yellow, moist, bright, round, sharply defined, convex and homogeneous. The appearance is the same by weak magnification. Deep colonies appear as white points.

Gelatine streak: Homogeneous colony along the inoculated line, the color of which is white at first but turns yellowish brown afterwards. Gelatine is not liquefied.

Gelatine stabculture: Thread like growth to the bottom.

Agar streak: Elevated, homogeneous, moist, dirty-white colony.

Potato: At 23°C. on the sixth day of inoculation a white elevated homogeneous colony is formed along the inoculated line.

Milk: Milk is coagulated, turning acid.

Oxygen: Aërobic.

Gas: Gas is not formed.

H_2S: H_2S is not formed.

Reduction: Nitrate is reduced to nitrite.

Acids: Acids are formed in glucose solution.

This micrococcus is the same as *Micrococcus I* of the silk-worm.

No. 5.

Form: The cell of 24 hour's culture in bouillon has a diameter of 1.2 μ. Two or more are united. It is colored by *Gram's* method.

Bouillon: At 23°C. on the second day it becomes turbid and on the fourth day a scum is formed and on the seventh day a white precipitate appears, the middle part being clear. It remains the same after 20 days.

Gelatine plate: Surface colony is yellow, round, sharply defined, granular, moist and bright. It appears alike by weak magnification. Gelatine is liquefied. Deep colony is a yellow point.

Gelatine streak: Sulphur-yellow, moist, homogeneous, elevated colony is formed along the inoculated line. Gelatine is quickly liquefied, yellow precipitate being formed.

Gelatine stabculture: Thread-like growth to the bottom, gelatine being liquefied at first in the form of a funnel but afterwards cylindrically.

Agar streak: Sulphur-yellow, moist, homogeneous, devated colony along the inoculated line.

Potato: At 23°C. dry, flat, white colony, having yellow granules thereon.

Millk: Milk is coagulated, acids being formed.

Oxygen: Aërobic.

Gas: Gas is not evolved.

H_2S: H_2S is formed.

Reduction. Nitrate is reduced to nitrite.

Acids: Acids one formed in glucose solution.

The yellow pigment is insoluble in water or alcohol, but soluble in potash solution. The pigment dissolved in the latter solution becomes colorless by the addition of HCl, which is restored again to yellow by alkali.

This micrococcus is a variety of *Micrococcus luteus, Lehmann et Neumann.*

No. 6.

Form: The cell cultured in bouillon for 24 hours has a diameter of 0.8 μ. Commonly two are united. It is colored by *Gram's* method.

Bouillon: On the third day a precipitate appears, and after 20 days a feeble yellow-brown scum and a yellow-brown precipitate are formed.

Gelatine plate: Surface colony is yellowish brown, moist, bright, round,

sharply defined and elevated. By weak magnification granular consistence is seen. Deep colony is a white point.

Gelatine streak: Light brown, tenacious colony is formed along the inoculated line. Gelatine is liquefied, film being formed.

Gelatine stabculture: Thread-like growth to the bottom, liquefying Gelatine in the form of a funnel.

Agar streak: Yellowish brown, moist, homogeneous, elevated colony.

Potato: At 23°C. on the second day a yellow colony is formed along the inoculated line. It assumes gradually a reddish yellow color and becomes elevated, dry and granular.

Milk: Milk is coagulated, showing an alkaline reaction.

Oxygen: Aërobic.

Gas: No gas is evolved.

H_2S: H_2S is formed.

Reduction: Nitrate is reduced to nitrite.

Indol reaction: A faint reaction.

Acids: 0.024% of acid, calculated from the dissolved Ca O as lactic acid, were formed in pepton-water containing 5% of glucose for a week at 15-20°C.

This micrococcus is probably *Micrococcus pyogenes aureus, Lehmann et Neumann.*

No. 7.

Form: The cell cultivated in bouillon for 24 hours has a diameter of 0.8 μ. Usually two are united, but sometimes four. It is colored by *Gram's* method.

Bouillon: On the second day it becomes turbid, and after 20 days a feeble ring on the wall, and a yellow precipitate were formed.

Gelatine plate: Surface colony is yellow, moist, bright, round, convex and sharply defined. By weak magnification it appears granular. Gelatine is liquefied. Deep colonies appear as white points.

Gelatine streak: Sulphur-yellow, homogeneous colony is formed along the inoculated line, gelatine being liquefied very quickly.

Gelatine stick: Thread-like growth to the bottom, liquefying gelatine in the form of a nail.

Agar streak: An elevated homogeneous, moist white colony is formed which gradually turns yellow.

Potato: At room-temperature elevated, yellow, moist bright, homogeneous colonies are formed along the inoculated line.

Milk: Milk is coagulated, acids being formed.

Oxygen: Aërobic.

Gas: Gas is not formed.

H_2S: H_2S is not formed.

Reduction: Nitrate is reduced to nitrite.

Acids: 0.013% of acid, culculated from the dissolved Ca O as lactic acid, is formed by cultivating in pepton-water containing glucose and Ca Co_3 for 14 days at 15-20°C.

The yellow pigment is insoluble in water or alcohol, but soluble in potash solution. The color is not destroyed by HCl or H_2SO_4.

This micrococcus is the same as *Micrococcus III* of the silk-worm, and is probably *Streptococcus bombycis* of *Macchiati*.

No. 8.

Form: The cell cultured in bouillon for 24 hours has a diameter of 1 μ. Commonly two are united but sometimes four. It is colored by *Gram's* method.

Bouillon: At 15°C. on the fourth day it becomes a little turbid, and on the sixth day a precipitate settles. It remains the same after 20 days.

Gelatine plate: Surface colony is white, round, elevated, sharply defined, and moist. By weak magnification it appears homogeneous. Deep colonies appear as white points.

Gelatine streak: An elevated, white, moist, homogeneous colony is formed. Gelatine is not liquefied.

Gelatine stabculture: Thread-like growth to the bottom.

Agar streak: A moist, bright dirty white, homogeneous colony along the inoculated line.

Potato: An elevated, white, moist, bright, homogeneous colony. On the central line it is more elevated.

Milk: Milk is coagulated, acids being formed.

Oxygen: Aërobic.

Gas: Gas is not formed.

H_2S: H_2S is formed.

Reduction: Nitrate is reduced to nitrite.

Acids: Acids are formed in glucose solution.

This is the same as *Micrococcus II* of the silk-worm, and is probably the *Streptococcus Pastorianus* of *Krassilschtschik*.

No. 9.

Form: The cell cultivated in bouillon for 24 hours is somewhat oblong and 0.8 μ. in the longer diameter. Usually two are united. It is coloured by *Gram's* method.

Bouillon: On the seventh day little white precipitate is formed, the fluid remaining clear. It is the same after 20 days.

Gelatine plate: Surface colony is deep yellow, round, elevated, sharply defined and moist. By weak magnification it appears granular. Deep colonies appear as yellow points. Gelatine is not liquefied.

Gelatine streak: Deep yellow, rather dry, homogeneous colony, which is much elevated on the central line. Gelatine is not liquefied.

Gelatine stabculture: Thread-like growth to the bottom.

Agar streak: It is the same as on gelatine, but the color is fainter.

Potato: At 23°C. on the fourth day flat, light yellow, moist, homogeneous colonies are formed along the inoculated line.

Milk: Milk is coagulated, acids being formed.

Oxygen: Aërobic.

Gas: Gas is not evolved.

H_2S: H_2S is formed.

Reduction: Nitrate is reduced to nitrite.

Indol reaction: A faint reaction.

Acids: 0.01% of acid, culculated as lactic acid, was produced in pepton-water containing 5% of glucose and some Ca CO_3 for 14 days at 15°-20°C.

This micrococcus is *Micrococcus aurantiaca, Cohn.*

No. 10.

The colony of this micrococcus is dark purple. The properties are not yet examined minutely.

Experiment XIV.

Nov. 11. 1902. On 10 A.M. 0.05 cc. of water in which the micrococci above described, cultured on agar, were suspended, were injected into silk-worms of the fifth day of the fifth stage, as in the former experiments. As control distilled water was also injected. All the larvæ except those that received water, lost appetite and on the next morning excreted liquid feces. They were kept in the sitting room and after Nov. 19 they were placed near a stove.

The number of the dead larvæ and the temperature during the experiment were as follows:—

	Number of the larvæ.	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Total.	% of the dead.
Temperature (c)[1] morning		14	11	11	18	16	11	9.5	11	20	13	18	20	20	20	20		
Temperature (c)[1] evening		22	—	—	24	20	13	—	18	—	18	25	20	20	20	20		
Control	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	13
Water	10	0	0	0	2	2	0	0	0	0	1	1	0	0	0	0	6	60
No. 1	10	0	0	0	3	0	0	0	0	1	0	1	2	0	0	0	7	70
No. 2	10	0	0	0	0	5												
No. 3	9	0	0	0	1	4	1	1	0	0	0	0	2	0	0	0	9	100
No. 4	10	0	0	0	1	6	0	1	0	0	0	1	0	0	0	1	10	100
No. 5	10	0	1	2	1	4	2										10	100
No. 6	8	0	0	0	2	3	1	0	0	0	0	1	1				8	100
No. 7	10	0	0	1	1	2	3	1	0	0	2						10	100
No. 8	10	0	0	0	0	1	3	0	0	0	2	2	2				10	100
No. 9	10	0	0	0	3	2	4	0	0	0	1						10	100
Micrococcus from the eggs	10	0	0	0	4	5	1										10	100

[1] Temperature after the 19th was very low during the night, as the stove was not used at night.

The conditions of the larvæ in each section of the experiment were as follows:—

Control.

15 silk-worms were kept for control. They were healthy and span cocoons on Nov. 25. During spinning two died of pebrine and flacherie. In the latter case many micrococci were found among green compressed fragments of mulberry-leaves. The average weight of the cocoons was 0.487 gr.

Water.

The larvæ recovered after a few hours.

Nov. 14. On the morning a larva died vomiting a yellow fluid, the body of which was shrunk. In the intestines large bacilli and diplococci were numerous. On the evening another died, fore-part of the body shrunk and back-part expanded. The fragments of mulberry-leaves in the intestines were green; here only large bacilli were found.

Nov. 15. On the morning one died, body softened and stretched out. Large bacilli were numerous, and also some diplococci were found. On the evening another died, body expanded in the middle part. The pieces of mulberry-leaves in the body were brown; streptococci were numerous.

Nov. 20. On the morning one died, body softened, back-part black. No fragments of mulberry-leaves present; some diplococci in the intestines.

Nov. 21. On the morning one died, body softened and stretched out. Some slender bacilli were present.

Nov. 25. The remainder (4) span coccoon, the average weight of which was 0.411 gr.

No. 1.

After the operation the larvæ lost appetite considerably.

Nov. 14. On the morning one died, the third and fourth segments elongated, excretion of soft brown dung of a faint acid reaction. The frag-

ments of mulberry-leaves in the intestines were not compressed; many large bacilli and some diplococci present. On the evening two died, one with shrunk fore-part and green compressed leaf-fragments, and bacilli and diplococci; the other with brown leaf-fragments and numerous bacilli of various size.

Nov. 19. On the evening one died, body softened and expanded in the middle part. Leaf-fragments not compressed; diplococci numerous.

Nov. 21. On the morning one died, body softened and expanded. The intestinal canal was filled only with liquid with numerous diplococci and few bacilli.

Nov. 22. On the morning two died, one with swollen segments and black back-part. In the intestines few leaf-fragments with some diplococci. The other with softened blackened body and green leaf-fragments; diplococci and bacilli present.

Nov. 25. Three remaining larvæ span cocoons, the average weight of which was 0.390 gr.

By preparing a plate-culture from the intestinal juice of the diseased larva many white colonies of the micrococcus inoculated besides various others appeared.

No. 2.

The larvæ lost appetite after the operation.

Nov. 13. On the morning five died. The first of them with some folds on the body; a few brown leaf fragments in the intestines and numerous diplococci.

The second also with folds, brown leaf-fragments and numerous diplococci and some streptococci. The third also with folds, brown leaf fragments, numerous diplococci and some streptococci. The fourth resembled the third; streptococci were more numerous, while with the fifth diplococci again prevailed, all the other conditions being the same.

Nov. 15. The remainder were unfortunately lost by an accident.

By preparing an agar-plate from the intestinal juice of the dead larva many colonies of the micrococcus inoculated were formed.

No. 3.

Nov. 14. On the evening one died, body softened and stretched, faint brown leaf fragments and large bacilli and diplococci in great number in the intestines.

Nov. 15. On larva died, body was faintly yellow, with some folds. Leaf fragments brown; diplococci numerous. On the evening three died, bodies softened and stretched. In the first the leaf fragments green, large bacilli and diplococci present. In the second brown leaf fragments, diplococci numerous. In the third no leaf fragments but a few diplococci were found in the intestines.

Nov. 16. On the morning one died, body stretched and leaf fragments green, diplococci numerous. All the other larvæ excreted liquid feces.

Nov. 17. On the morning one died, fore-part of body somewhat transparent, back-part thin. The few leaf fragments green, few diplococci and pebrine-organisms present.

Nov. 22. On the morning two died, both with softened body and many diplococci, the one with brown leaf fragments; the other with empty intestines, the back part of the latter larva was black.

No. 4.

The larvæ showed poor appetite after the operation.

Nov. 14. On the morning one died, body rather hard, leaf fragments brown and compressed, diplococci numerous.

Nov. 15. On the morning three died, fore-part of bodies shrunk. Leaf fragments brown, numerous diplococci. On the evening three died, bodies softened, leaf fragments green, diplococci numerous.

Nov. 17. On the morning one died, body softened, leaf fragments green, streptococci numerous.

Nov. 21. On the morning one died, leaf fragments compressed; no bacteria were observed.

Nov. 25. One died, body softened. Few fragments of leaves, many diplococci.

By preparing an agar-plate from the dead larva numerous light brown colonies of diplococci were formed.

No. 5.

The larvæ lost all appetite by the operation.

Nov. 12. At noon one died, excreting a brown fluid of a faint acid reaction from the anus. The intestinal canal was full of brown fragments of leaves; numerous diplococci present.

Nov. 13. On the morning two died, excreting a brown fluid of a faintly acid reaction from the anus. One contained many large bacilli and few diplococci; the other few small bacilli and micrococci.

Nov. 14. On the morning one died vomiting a brown fluid, the third and fourth segments were elongated, the middle part of the body expanded, black lines appearing on the fourth and fifth segment, leaf fragments brown and compressed, numerous diplococci and some large bacilli present.

Nov. 15. On the morning four died, bodies stretched and containing brown leaf fragments. In the first the back part of the body black and streptococci numerous. In the three others streptococci were numerous.

Nov. 16. On the morning two died, bodies softened. Leaf fragments brown, diplococci numerous.

By preparing an agar-plate from the intestinal juice of the dead larva yellow colonies of diplococci were produced in large number.

No. 6.

The larvæ lost completely appetite by the operation.

Nov. 13. On the mording one excreted a light yellow fluid of a faintly acid reaction from the anus.

Nov. 14. On the morning two died. One with stretched and softened body, and green leaf fragments. The second with shrunken body had excreted a yellow fluid of a faintly acid reaction from the anus. Leaf fragments green, numerous diplococci present.

Nov. 15. On the morning two died, bodies faintly yellow. In one of them leaf fragments were few and compressed; diplococci and streptococci numerous. In the other leaf fragments brown, numerous streptococci and some large bacilli present. On the evening one died, the middle part expanded, leaf fragments brown and compressed, diplococci numerous.

Nov. 16. On the morning one died, body was softened, leaf fragments green, diplococci numerous.

Nov. 21. On the morning one died, body shrunk, diplococci present in large number.

Nov. 22. On the morning one died, the fore-part shrunk, and back part blackened. Leaf fragments brown, numerous diplococci present.

No. 7.

Nov. 13. On the morning one died, body shrunk, leaf fragments brown and compressed. There were found a few micrococci.

Nov. 14. On the morning one died, body softened, leaf fragments brown, many large bacilli and few diplococci present.

Nov. 15. On the morning two died, one with softened body and few green leaf fragments, streptococci numerous. In the other leaf fragments were brown and compressed; numerous micrococci and few streptococci present.

Nov. 16. Three died, bodies expanded in the middle part. In the first leaf fragments green, large bacilli present. In the second the leaf fragments brown, streptococci and large bacilli numerous. In the third the leaf fragments brown, diplococci numerous.

Nov. 17. On the morning one died, body softened and leaf fragments brown and compressed, diplococci numerous.

Nov. 20. On the morning two died; one with softened and faintly purple-colored bodies, leaf fragments brown and compressed, bacilli numerous. In the other body softened, the back part blackened, leaf fragments brown. Some *saccharomycetes* and large bacilli were found.

No. 8.

Nov. 15. On the morning one died, body expanded in the middle part, leaf fragments green, streptococci numerous.

Nov. 16. On the morning three died. In one the middle part of the faintly yellow colored body expanded, leaf fragments brown with many diplococci. In the second body softened, leaf fragments green and compressed, short bacilli numerous. In the third body softened, leaf fragments green, numerous streptococci present.

Nov. 20. On the morning two died, with softened bodies and brown leaf fragments; diplococci numerous; in one also bacilli present.

Nov. 21. On the morning two died, one with contracted fore-part and a few diplococci; the other with the fore-part elongated and many diplococci.

Nov. 22. On the morning two died during the spinning of cocoon; bodies softened, the back part black, leaf fragments green. In one bacilli were numerous but diplococci few; in the other bacilli and diplococci were equally numerous.

By preparing an agar-plate from the intestinal juice of the dead larva only white colonies of micrococci were formed.

No. 9.

Nov. On the morning three died, two with the body shrunk, and one with the body elongated. One with many bacilli and few diplococci, and brown leaf fragments; the second with numerous diplococci, and green compressed leaf fragments; the third with brown leaf fragments, numerous diplococci and some large bacilli.

Nov. 15. On the morning one died, body black, leaf fragments brown, diplococci numerous but streptococci few. On the evening one died, body softened, leaf fragments green, diplococci numerous.

Nov. 16. On the morning four died, bodies, softened. With three of them small black spots appeared on the body; intestinal contents were

green. In one of these diplococci, streptococci and some pebrine-organisms were observed. With the second large bacilli and diplococci. With the third streptococci. With the fourth green compressed leaf fragments, and streptococci numerous, large bacilli few.

Nov. 20. On the morning one died, body softened with small black spots all over. Leaf fragments brown and compressed, diplococci numerous.

By preparing an agar-plate from the intestinal juice of the dead larva, yellow colonies of diplococci were exclusively formed.

Micrococcus from the eggs.

Nov. 14. On the morning two died, in one the body stretched, the second and third segments yellow, and leaf fragments brown and compressed, few diplococci were observed. In the other the body was shrunk and hard, leaf fragments also brown and compressed; sarcina was found exclusively. On the evening two died, leaf fragments brown and compressed, diplococci present.

Nov. 15. On the morning five died, bodies softened with brown leaf fragments and numerous diplococci in every case.

Nov. 16. One died, body softened, leaf fragments brown, few diplococci.

By preparing an agar-plate from the intestinal juice of the dead larva, yellow colonies of sarcina were exclusively formed.

From the results of these experiments the following conclusions were drawn.

1. The micrococci on the mulberry-leaves can cause flacherie, *No. 1* being the least able to multiply in the intestines of the silk-worm.

2. Flacherie can be caused by this micrococcus, what can be proved by the fact that by preparing a plate-culture from the diseased larvæ the colonies of the inoculated micrococcus were formed in greater number or exclusively.

3. Since flacherie is caused by injecting water into the intestines, it is clear that the bacteria that can cause flacherie exist always in the intestinal canal, which fact proves also that the mulberry-leaves are the carriers of the germs.

4. The micrococcus in the eggs can cause flacherie.

5. The decrease of appetite of the silk-worm by the injection of water or bacteria can be observed also from the diminished weight of the cocoons formed.

	Average weight of a cocoon.
Control	0.487 gr.
Water injected	0.411
Micrococcus No. I inoculated	0.390

6. Any constant relation between the bacteria inoculated and the symptoms of the malady was not observed in these experiments.

7. When mulberry-leaves in the intestines are green, the reaction of the juice is alkaline, though weaker than in the healthy animal, while the brown color indicates that the reaction is neutral or faintly alkaline.

8. *Bac. megatherium* seems to multiply usually after the micrococci had developed luxuriantly.

9. At low temperature the bacteria do not bring on flacherie very soon.[1] The cause will probably be due to the slow growth of the bacteria at the low temperature.

General Conclusions.

From the results of the series of the experiments above described the following conclusions were drawn.

I. There is no doubt that flacherie is caused by the growth of bacteria in the intestinal juice.

II. The bacteria usually found in large number in the intestinal juice of the diseased larvæ are various kinds of micrococci and two kinds of bacilli. In most cases micrococci only or together with few large bacilli are found. A short bacillus is also usually found along with the micrococci; however cases in which the short bacillus alone is found are very rare. Besides these microbes there are many other kinds which exist in a small number in the diseased animals.

[1] Compare Experiments VII and XII.

III. The large bacillus was identified with *Bacillus megatherium, De Bary*, and the short one with the coli-bacillus.

IV. There exists in the interior of the eggs of the silk-worm usually a micrococcus and a large bacillus. The former was identified with *Sarcina lutea, Flügge* and the latter with *Bacillus megatherium, De Bary*.

V. Various kinds of micrococci usually adhere to the mulberry-leaves. The writer isolated from mulberry-leaves 10 species of micrococci. Nine of them were used for the experiments, by which it was decided that flacherie is caused by these micrococci and the sarcina isolated from the eggs. The micrococci isolated from the dead larvæ were identified with those isolated from mulberry-leaves.

VI. It is clear that the sources of the bacteria, which multiply in the intestines of the silk-worm and cause flacherie, are the mulberry-leaves serving as food. But when the larvæ are healthy they resist the action of the bacteria. However when silk-worms are reared at high temperature or any disorders are produced in the digestive organs, the microbes multiply and cause the malady. The greater number of micrococci in the intestinal juice is due to their abundance also on mulberry-leaves.

VII. Flacherie is, as above explained, not caused by any special bacteria, hence *Macchiatis'* and *Krassilschtschik's* assumption can not be comfirmed. My observations agree with those of the Austrian Experiment Station that flacherie is not infectious.

VIII. The true cause of the disease is the increase of certain products formed by the undue and rapid multiplication of various microbes. These products are in all probability no toxins, but they may consist of ammonia formed by protein decomposition, or of nitrite formed from nitrate contained in the leaves, or of acids produced from carbohydrates. Very probably these noxious substances are sometimes acting together. I hope to settle this question satisfactorily by further investigations.

The author must express here his sincere thanks to *Prof. Sasaki* who kindly translated Italian articles for him, further to *Prof. Loew* and *Prof. Kozai*, and to *Mr. Honda* and *Mr. Hayashi*, Experts of Tokio Sericultural Institute who furnished him the larvæ and eggs of the silk-worms, and finally to *Mr. Yamasaki*, Assistant of the College.

Zur Physiologie des Bacillus pyocyaneus, II.

VON

O. Loew und Y. Kozai.

In Fortsetzung unserer früherer Versuche,[1] eine möglichst günstige Nährstoffloesung für den *Bac. pyocyaneus* zu finden, in welcher trotz lebhafter Vegetation keine Schleimbildung aber reichliche Enzymbildung statthabe, fanden wir folgende Lœsung diesen Bedingungen entsprechend:

Pepton.	0.5 %
Glycerin.	0.1 „
Magnesiumsulfat.	0.01 „
Dikaliumphosphat.	0.1 „
Natriumbicarbonat.	0.1 „
Chlornatrium.	0.4 „

Das Magnesiumsulfat wurde sterilisirt bei der Infection zugesetzt. Wir vaviirten in dieser Lœsung die einzelnen Bestandteile mehrfach und jedesmal war das Resultat entweder eine langsamere Vegetation oder eine Verzögerung der Wiederauflœsung der Bacterienmassen.[2] In dieser Lœsung läuft die Vegetation in 18—20 Tagen bei 25—28°C. ab, wenn die Kolben *nur zur Hälfte* voll sind was behufs reichlicher Enzymproduction nötig ist und jeden Tag kräftig ungeschüttelt wird, wobei unter Sauerstoffabsorption die gelbe Lœsung tief grün wird. Est vom 13. Tage ab hört die Reduction des grünen Farbstoffs auf. Die anfänglich reichlichen Bacterienmassen lösen sich bis auf einen geringen Bodensatz allmälig wieder auf.

[1] Siehe diese Bulletins, Bd. 4, No. 4 und No. 5.

[2] Wir erhöhten z. B. das Pepton auf 1%, das Dikaliumphosphat auf 0.4%, wir eliminirten das Natriumbicarbonat und setzten endlich die Chlornatriummenge auf 0.2 und 0.1% herab; auch versuchten wir dieses durch Natriumsulfat zu ersetzen.

Diese zuerst von *Emmerich* und *Loew* beobachtete Wiederaufloesung wurde von *Conradi* als eine Antolyse aufgefasst, was aber wohl nicht dem wirklichen Vorgange entspricht; denn Autolyse[1] ist die Gesammtheit der in einem Organ (oder Organismus) nach dem Tode stattfindenden fermentativen Vorgänge, es wird also als characteristisch angesehen, dass *irgend eine vorherige Secernirung von Enzym nicht stattfindet*. Andernfalls ist der Vorgang eben lediglich eine gewöhnliche Verdauung; denn es ist doch z. B. ganz und gar irrelevant, ob ein Magensecret den Magen verdaut, der es abgesondert hat, oder einen anderen Magen. Bei der Wiederaufloesung der gewachsenen Bacterienmassen durch die secernirte Pyocyanase muss erst eine gewisse Anhäufung der letzteren in der Culturflüssigkeit erreicht worden sein. Dann erst kann der Angriff auf die Nucleoproteide[2] der Bacterienleiber Erfolg haben und wird dann auch das weitere Wachstum eingeschränkt und endlich ganz verhindert.[3] Wenn aber die Bacillen auf festem Nährboden (Glycerin-Agar) cultivirt werden, so bleibt jedenfalls das Enzym in den Zellen, wenigstens grossenteils. Dafür spricht die Beobachtung von *Krause*,[4] dass der Presssaft des *B. pyocyaneus* milzbrandheilend wirkt.[5] Die Bacillen waren auf Agarplatten cultivirt und die Vegetation nach 48 Stunden mit Platinspatel abgenommen worden. Nebenbei bemerkt muss dieser Presssaft kaum Toxin und auch nur wenig Pyocyanolysin enthalten haben; denn 3 cc. waren nach *Krause* einem Kaninchen nicht schädlich.

Bei dem Interesse, welches sich an die Pyocyanase knüpft, suchten

[1] *Theobald Smith* hat bereits i. J. 1894 die verdauende Wirkung in sterilen Geweben von Thieren beobachtet; später haben *Salkowsky*, *Jacobi*, *Mignus-Levy*, diese Erscheinung weiter verfolgt. Besonders interessant sind die Resultate Conradi's.

[2] *Krawkow* (*Hofmeisters* Beiträge I, 530) hat Pyocyaneus-Zellen mit verdünnten Natron extrahirt, mit Essigsäure die Loesung gefällt und nach dem Reinigen das Nucleoproteid analysirt. Er fand darin: C=52.73%; H=6.91%; N=16.50%; P=2.11%; S=1.0%. In den Membranen fand er C=46.2%; H=6.7%; N=8.8. Dieser Stickstoffgehalt deutet auf eine chitinartige Substanz, was auch von *Emmerling* für die Membranen des Bac. fluorescens liquefaciens vermutet wird (Ber. Chem. Ges. *35*, 702).

[3] Nach *Siegwart* (C. Bakt. *30*, 573) werden die Nucleoproteide der Bacterien auch von Pepsin verdaut—aber erst nachdem die Bacillen getödtet sind, was jedenfalls auffallend ist.

[4] Centrbl. f. Bakt. *31*, No. 14.

[5] Typhus konnte beim Meerschweinchen damit nicht geheilt werden.

wir nach einer Methode, welche bei grosser Einfachheit doch ein reineres Product liefert, als bisher möglich war. Unser Ziel ist noch nicht erreicht worden, doch mögen immerhin einige Beobachtungen der Mittheilung wert sein. Vor einigen Jahren hat *F. Neuenberg*[1] über erfolgreiche Behandlung der Staphylomykosis mit der Pyocyanase (Rohfermentloesung) berichtet. Derselbe stellte, wie *K. Vaerst*[2] in seinen erfolgreichen Versuchen der Mitzbrandbehandlung mit Pyocyanase, dieselbe in etwas verschiedenen Weise dar, wie *Emmerich* und *Loew*, nämlich durch Aussalzen nach Erhitzen auf 58° (6 Stunden). Auf 1 L. der sechswöchentlichen Bouilloncultur wurden 500 g. Ammonsulfat gegeben,[3] nach 24 Stunden das Ausgeschiedene einer mehrtägigen Dialyse überlassen und dann die Loesung im Vacuum zur Trockne gebracht. Die alkalische Loesung wurde also nicht erst neutralisirt, und in der Tat haben uns vergleichende Versuche gezeigt, dass dieses vorzuziehen ist. Beim Versetzen mit Essigsäure[4] wird Kohlensäure frei, welche beim nachfolgenden Aussalzen in Blasen festgehalten wird, so dass eine sehr schaumige Masse erhalten wird, welche schweer weiter zu behandeln ist. *Neuenberg* sowohl wie *Vaerst* verwendeten Bouillonculturen. Diese liefern aber eine sehr schleimige Flüssigkeit,[5] welche beim Aussalzen auch den Schleim ausscheidet, der nun einen grösseren oder geringeren Theil der Pyocyanase mit sich reisst. Wird nun diese Ausscheidung der Dialyse unterworfen, um das Ammonsulfat zu entfernen, so bemerkt man eine auffallende Abnahme des Schleims, so dass man zur Vermutung kommt, es habe ein mit ausgeschiedenes Enzym (wegen nun grösserer Concentration) den Schleim durch Hydrolyse in nicht schleimige Producte verwandelt. Wenn die dialysirte Loesung dann im Vacuum eingedampft wird, so wirkt kein Schleim mehr störend, beim Loesen, resp. Injiciren des Products. Wir haben aus 1 Liter Bouilloncultur

[1] Habilitationsschrift, Bern 1900.

[2] Centrbl. f. Bakt. *31*, No. 7.

[3] Eine mässige Vermehrung des Salzes bringt nur noch eine geringe Mehrausscheidung zu Wege.

[4] Es ist nahe zu 1 promille Essigsäure behufs Neutralisation nötig.

Diese Schleimbildung beruht vielleicht auf der Gegenwart milchsaurer Salze. Auch essigsaure Salze und Asparagin liefen schleimige Culturen, Pepton aber nicht.

nur 1.5 g. des Rohferments erhalten. Prof. Nitta beobachtete, nach Darreichung von 0.1 g. desselben *per os*, bei einem Meerschweinchen keine Spur eine Temperaturerhöhung oder irgend welchen andern Effect, es waren also keine Substanzen vorhanden, die *per os* hätten schädlich wirken können, was von einigem Interesse sein mag, falls einmal dieses Rohferment zur Bekämpfung von Bacillen (Cholera) im Darm zur Verwendung kommen sollte.

Wir haben nun die Aussalzmethode auch bei Culturen angewandt, welche *nicht schleimig* werden, speciell bei der eingangs erwähnten Culturloesung. Zehn Liter der 18 tägigen Cultur wurden zunächst mit Chloroform versetzt und einen Tag stehen gelassen, um etwa noch vorhandene lebende Zellen abzutöten. Am folgenden Tage zeigte die Flüssigkeit einen intensiven *Geruch nach Isonitril*, es musste also ein primäres Amin in der Cultur gebildet worden sein. Die klare Loesung wurde abgegossen, der letzte Theil filtrirt und in die Gesammtmenge der alkalisch reagirenden Flüssigkeit (10 L.) sechs Kilo Ammonsulfat eingetragen und unter häufigen Umrühren bei 6—8° stehen gelassen. Es schied sich nach einiger Zeit eine flockige Masse an der Oberfläche ab, welche abgenommen und durch Filtration und Pressen von der anhängenden Ammonsulfatloesung so gut wie möglich getrennt wurde. Durch dreitägige Dialyse wurde der Rest des Ammonsulfats entfernt. Schon beim Anrühren mit Wasser wurde bemerkt, dass sich ein grosser Teil nicht wieder löste, trotzdem wurde die Gesammtmasse in den Dialysirschlauch[1] gegeben. Der unlösliche Theil war von einer melaninartigen Substanz schwarz gefärbt, und enthielt neben blaugrünen Pyocyaneusfarbstoff noch einen geringen Anteil höherer Fettsäuren, und etwas Proteinsubstanz. Das Filtrat wurde zunächst auf verdauende Wirkung geprüft, aber nur eine äusserst schwache Wirkung auf gequollenes Blutfibrin beobachtet, selbst als noch 0.2% Soda zugesetzt wurde. Daraus durfte wohl der Schluss gezogen werden, dass die Pyocyanase keine Albumosenatur besitzt,[2] sonst wäre sie mit ausgesalzen worden. Bei den Versuchen von *Neuenberg* und von *Vaerst* musste wohl die volumi-

[1] Zu antiseptischem Zwecke wurde auch etwas Chloroform zugesetzt.

[2] Wahrscheinlich ähnelt sie den Peptonen.

nöse Schleimmasse, die ausgesalzen wurde, viel Enzym mit niedergerissen haben.

Es ist desshalb wohl der Schluss gerechtfertigt, dass bei nicht schleimigen Culturen die Abdampfmethode (im Vacuum) der Aussalzmethode vorzuziehen ist, da sie sicher die Gesammtmenge des Enzyms liefert.

Über den Kalkgehalt der Milchdrüse.

VON

M. Toyonaga.

Ich habe in meiner früheren Arbeit über den Kalkgehalt der grauen und weissen Hirnsubstanz darauf hingewiesen, dass die Drüsen im Verhältnis zur Magnesia viel mehr Kalk enthalten als andere Gewebe des Tierkörpers, was jedenfalls mit der grösseren Zellkernmasse zusammenhängt. Es war in dieser Beziehung natürlich von Interesse diese Untersuchungen fortzusetzen, insbesondere weil in Bezug auf die verschiedenen Organe des Tierkörpers auffallend wenige Aschen-Analysen vorliegen, während in Bezug auf den Pflanzenkörper diese äusserst zalreich sind.

Ich habe zunächst die Milchdrüse in Betracht gezogen, welche insbesondere deshalb Beachtung verdient, weil ihr Secret in Bezug auf Mineralbestandtheile ganz ausserordentlich von dem Blute differiert; so fand *Bunge:*—

100 Theile Asche enthalten:	Hundemilch.	Hundeblut.
K_2O	10,7	3,1
Na_2O	6,1	45,6
Ca O	34,4	0,9
Mg O	1,5	0,4
Fe_2O_3	0,14	9,4
P_2O_5	37,5	13,3
Cl	12,4	35,6

Wir ersehen hieraus in Bezug auf den Kalkgehalt ganz enorme Unterschiede. In der Hundemilch berechnet sich das Verhältnis

$$MgO : CaO = 1 : 22{,}93$$

in Hundeblute $\quad MgO : CaO = 1 : 2{,}25$

Ich beschränkte mich bei der Analyse auf die Bestimmung des Kalks und der Magnesia, da besonders dieses Verhältnis für die verschiedenen Gewebe sehr charakteristisch, und die Asche der Milchdrüse überhaupt noch nicht untersucht ist.

Ich trennte bei der Milchdrüse einer Kuh so gut als möglich das Bindegewebe von der eigentlichen Drüsensubstanz ab und bestimmte zunächst den Wassergehalt, derselbe betrug 66.7%.

Nun wurden 88,641 g Trockensubstanz mit 5 g wasserfreiem Natriumcarbonat gemischt und verascht wobei das Weissbrennen wie gewöhnlich sehr lange dauerte. Die Masse wurde zunächst mit Wasser extrahiert und nach Entfernung des kohlensauren- und phosphorsauren Natrons der ausgewaschene Rückstand mit Salzsäure gelöst, wobei eine Minimalmenge Kieselsäure ungelöst blieb hierauf die Lösung mit Ammoniak bis zu alkalischer Reaktion versetzt und dann mit Essigsäure bis zu schwach saurer Reaktion vermischt.

Hierbei bleibt ein geringer flockiger Niederschlag von phosphorsaurem Eisen ungelöst. Aus dem Filtrat wurde nun der Kalk mit oxalsaurem Ammoniak gefällt und das eingeengte Filtrat vom Kalkniederschlag zur Magnesiabestimmung verwendet.

Es wurde erhalten:

$CaCO_3$ = 0,3995 g = 0,2231 g CaO

$Mg_2P_2O_7$ = 0,1562 g = 0,0566 g MgO

hieraus berechnet sich für 1000 Teile frischer Drüse:	100 Teile der Trockensubstanz:
CaO = 0,8401 Teile	0,2517 Teile
MgO = 0,2131 „	0,0639 „

Vergleichen wir das sich hieraus ergebende Verhältnis $\frac{Ca}{Mg}$ mit den für Milz und Niere von Aloy[1] gefundenen Zahlen und mit den Zahlen für das Muskelfleisch von Säugetieren, so ergiebt sich:

	Milchdrüse.	Milz.	Pankreas.	Niere.	Säugetier-Muskel.
$\frac{Ca}{Mg}$	4,67	6,79	4,05	1,84	0,34

Es ist somit auch bei der Milchdrüse wie bei der anderen Drüsen der

[1] Jahresbericht f. Thierchemie 30, S. 492.

Calciumgehalt grösser als der Magnesiumgehalt, während für das Muskelgewebe der Warmblüter umgekehrt der Magnesium gehalt grösser ist als der Calciumgehalt.

Vergleichen wir noch die Mengen von Ca und Mg im Muskel mit denen in Milchdrüse und Milz, so hat man für die organische Trockensubstanz:

Säugethier *Muskel* (Katz).	*Milchdrüse.*	*Milz* (Rind)
Ca ——— 0.033%	0.173%	0.141%
Mg ——— 0.109 „	0.038 „	0.056 „

Es ergibt sich somit, dass nicht nur der Calciumgehalt absolut grösser ist in der Drüse wie im Muskel, sondern auch dass der Magnesiumgehalt dort weit geringer ist als hier. Ich werde meine Untersuchungen fortsetzen.

Der Erntequotient.

VON

Oscar Loew.

In allen vollständigen Ernteberichten aus der Praxis sowohl, wie den Versuchs-Stationen, wird ausser dem wesentlichen Erntebestandteil, wie Knollen, Wurzeln, Früchten auch noch die Menge des Krautes oder Strohes angegeben. Aus diesen Zahlen ersieht man aber nicht sofort, ob sich das Verhältniss zwischen diesen Bestandteilen dem Mittel oder einem Optimum nähert. Behufs einer sofortigen Beurteilung dieses Verhältnisses möchte ich den Begriff des *Erntequotienten* einzuführen vorschlagen. Er gestattet sofort zu ersehen, ob unter den gegebenen Bedingungen (Boden, Düngung, Wetter, etc.) ein mittleres oder optimales Verhältniss erzielt wurde. Er gibt die Hauptleistung der Blätter, der wichtigsten Producenten organischer Materie, in vergleichbaren Zalen an, er zeigt, ob diese Organe ihre Aufgabe voll und ganz erfüllt haben. Dieser Erntequotient

$$q = \frac{k}{s} \cdot 100$$

drückt die Ernte des wesentlichsten Bestandteils k = Körner, Knollen, Wurzeln, in Procenten der Blattsubstanz, des Stroh's, s, aus. Man kann für die Zwecke der Praxis die Gewichte des lufttrocknen Krautes oder Strohs zu Grunde legen, während für rein wissenschaftliche Zwecke das Gewicht der absoluten Trockensubstanz zu dienen hätte. Es wäre von einigem Vorteil, den absoluten Erntewerten pro ha auch den Erntequotienten, der sich bei normalen Pflanzen oft zwischen genau bestimmten Gränzen bewegt, beizufügen. So beträgt derselbe im Mittel bei Gerste 73, während er im Optimum, wie es wohl in der Praxis nicht erreicht wird, 100 betragen kann.[1] Bei Bohnen liegt er in der Regel weit über 100, bei Erbsen häufig-über 200.

[1] *Hellriegel* teilt mit, dass er unter sehr günstigen Bedingungen im Glashaus bei Gerste gleiche Gewichte Stroh und Körner geerntet habe. Andererseits beschreibt *E. Wolff* Feldversuche, welche auf 100 Stroh nur 60 Thl. Körner gaben und bei Weizen gar nur 40.

Ferner würde er sich im Mittel aus zalreichen Daten ergeben für

Weizen	53
Hafer	66
Mais	80
Senf	52
Buchweizen	54

Wohl haben schon verschiedene Forscher den Körnerertrag hie und da auf 100 Theile Stroh bezogen, aber es ist systematisch weder der mittlere noch der optimale „*Erntequotient*“ bestimmt worden. Besonders war es *P. Wagner*, welcher seine Resultate mit Cerealien in dieser Form ausdrückte. So fand er z. B. dass bei verschieden starker Stickstoffdüngung auf 100 Thl. Stroh resultiren können bei Hafer 51–87 Thl. Körner, bei Roggen 46–53, bei Weizen 33–63. Ferner hat er bei Hafer auf 100 Thl. Stroh 52 Thl. Körner erhalten, als er Chilesalpeter bei der Einsaat gab, aber 64 Thl. Körner, wenn er diesen bei beginnendem Schossen zufügte.[1]

Gewisse Verhältnisse führen zu einem Uebermass von Blattproduction, andere wieder ermöglichen den Blättern, die von ihnen bereiteten organischen Nährstoffe in ausgiebigster Weise der Ausbildung der Früchte zukommen zu lassen. Diese Arbeit mit einer Zal auszudrücken, beabsichtigt der *Erntequotient*.

[1] Die Stickstoffdüngung der landwirtschaftlichen Culturpflanzen, 1892, S. 164.

Ueber die physiologische Wirkung des Chlorrubidiums auf Phanerogamen.

VON

Oscar Loew.

Versuche mit Buchweizen hatten mir früher gezeigt,[1] dass eine physiologische Vertretung von Kalium durch das ihm so nahe stehende Rubidium nicht möglich ist. Zu diesem Schlusse zwar schon vor mir *Birner* und *Lucanus*[2] gekommen, allein ich constatirte immerhin einen grossen Unterschied zwischen der Wirkung von Rubidiumnitrat und Rubidiumchlorid. Mit Nitrat ergaben sich pathologische Stärkeanschoppungen, eine Verdickung und Torsion des Stengels, Sistirung des Längenwachstums, Einrollen und Fleischigwerden der Blätter und schliesslich erfolgte der Tod, bevor eine Blüte entwickelt war. Wurde gleichzeitig ein Chlorid (Salmiak) zugesetzt oder Rubidium nicht als Nitrat, sondern als Chlorid verwendet, so streckten sich die Pflanzen und gelangten nach Erreichung einer weit bedeutenderen Höhe bis zur Blütenbildung was deutlich für den Einfluss von Chloriden auf den Stärketransport spricht. Erst nach der Blütenbildung traten Hemmungserscheinungen ein, es fand eine Anhäufung von Zucker und Veränderungen des Chlorophylls statt und die Pflanzen verfielen einem langsamen Siechtum, ohne einen Samen producirt zu haben. Weiter gelangten Pflanzen, denen Kalium und Rubidium zugleich gegeben wurde, indem die Hälfte des in der Controlloesung verwendeten Chlorkaliums durch Chlorrubidium ersetzt war. Indessen auch hier wurde die Höhe der Controlpflanzen nicht erreicht und kein reifer Same gebildet, die Pflanzen starben nach der Blütenperiode ab. Trotz der pathologischen Wirkungen ergab sich immerhin

[1] Landw. Vers. Stat. *21*, S. 389.

[2] Ibid. 7, S. 263.

für das Rubidium ein physiologischer Nutzen, den das Natrium nicht besass, denn die Pflanzen producirten weit mehr Trockensubstanz, was vielleicht nur auf einer Unterstützung der Wirkung der im Samen gespeicherten Kaliumsalze beruhen mag. Von Interesse ist hier die Beobachtung von *Molisch*,[1] dass Algen in einer Culturloesung sich gar nicht entwickeln, wenn darin statt der Kaliumsalze Rubidiumsalze vorhanden sind. Ist es hier die Zellteilung oder die Assimilation des Kohlenstoffs, oder die Eiweissbildung oder sind es diese drei wichtigsten Vorgänge zusammen, welche mit Rubidium statt des Kaliums nicht ausgeführt werden können? Diese Frage konnten vielleicht Versuche mit Pilzen entscheiden. Hier beobachtete ich nun, dass Bierhefe und der gemeine Pinselschimmel sich sogar noch besser entwickeln können, wenn bei Zucker als organischer Kohlenstoffquelle Rubidium statt des Kaliums dargeboten wird. Beide Elemente kamen als Tartrate zur Verwendung. Werden jedoch weniger gute Kohlenstoffquellen, wie Natriumacetat, verwendet, so stösst man auf einen bedeutenden Unterschied zu Gunsten des Kaliums. Da nun verschiedene Pilze eine verschiedene Wachstumsgeschwindigkeit besitzen, somit wahrscheinlich der Eiweissbildungsprocess mit ungleicher Fertigkeit ausgeführt werden dürfte, liess sich vermuten, dass die Verwendbarkeit des Rubidiums bei diesem Process auch nicht stets mit derselben Leichtigkeit vor sich gienge. In der Tat hat Günther[2] beobachtet, dass während der Pilz *Botrytis cinerea* Rubidiumsalze physiologisch verwerten kann, dieses bei *Rhizopus nigricans* nicht der Fall ist. Ich beobachtete eine Vertretbarkeit bei *Bacterium coli* und, wenn auch in weit geringerem Grade, bei *B. pyocyaneus*, während bei *Cladothrix odorifera* selbst bei Zucker als Nährstoff eine Vertretung sich als unmöglich erwies.

Rubidiumsalze zeigen somit in physiologischer Beziehung ein eigenartiges Verhalten. Die oben erwähnten pathologischen Effecte beim Buchweizen einerseits, die günstigen Effecte bei Hefe und Schimmel andererseits veranlassten mich, die Versuche mit Phanerogamen in modificirter Form wieder aufzunehmen. Ich versuchte die Wirkung kleiner Dosen Rubidiumchlorids bei Pflanzen unter normalen Ernährungsbedingungen.

[1] Wien. Akad. Ber. 1896.

[2] Inauguraldissertation, Erlangen 1897.

Versuch mit Brassica chinensis.

Drei Töpfe mit je 1 Kg. Boden wurden gedüngt mit: 1 g. Kaliumnitrat, 0.5 g Ammonsulfat, und 0.5 g. Monokaliumphosphat. Ausserdem erhielt

Topf a, 10 Milligramm Rubidiumchlorid.

„ b, 50 „ „

„ c. diente zur Controlle.

Der Chlorgehalt des Bodens entsprach nahe zu 0.05 g. Na Cl per Kilo, er war ein lehmiger Boden, zum Teil aus vulkanischer Asche bestehend.

Am 21, October wurden 10 Samen pro Topf ausgesät und am 5. November die jungen Pflanzen auf je drei möglichst gleich grosse, 6-7 cm. hohe, reducirt. Gegen Mitte November ergab sich, mit Ausnahme einer Pflanze in b für die Rubidiumpflanzen ein besseres Wachstum als für die Controlpflanzen, ein Unterschied, der mit der weiteren Entwicklung immer bedeutender wurde. Am 17. December ergaben die Messungen für das längste Blatt jeder Pflanze Folgendes: —

a	b.	c. Controlpflanzen.
21.0 cm.	14.0	16.5
22.4 „	19.1	17.0
28.2 „	25.5	21.1

Am 22. Dec. wurden die Pflanzen ausgezogen, die Wurzeln gereinigt und mit Fliesspapier gut abgetrocknet, und die ganzen Pflanzen gewogen im frischen Zustande, mit folgendem Ergebniss: —

a	b.	c.
14.3 g.	6.1	10.1
16.7 „	14.0	10.2
18.8 „	25.2	15.0
Mittel: 16.6	15.1	11.8

Es war somit ein stimulirender Effect des Rubidiumchlorids zweifellos, doch war dieser bei Erhöhung von 10 Milligramm auf 50 pro Kg. Boden nicht vermehrt worden, die Pflanzenmasse war im Gegenteil in letztrem Falle etwas kleiner als im ersteren.

Versuch mit Gerste.

Zwei Töpfe mit je 1 Kg. lufttrocknem Boden erhielten als Grunddüngung je 1.5 g. Ammoniumsulfat, 0.5 g. Monokaliumphosphat, 1.5 g. Calciumsuperphosphat, 1.0 g. Kaliumcarbonat[1] und 0.5 g. Natriumnitrat. Einer erhielt ausserdem noch 0.2 g. Rubidiumchlorid, der andere die aequivalente Menge Natriumchlorid. In jeden Topf wurden am 14 October 10 vorher gequollene Samen ausgesät und die Entwicklung im Glashause wie beim vorigen Versuch beobachted. Am 21. October wurden die Pflanzen auf 4 pro Topf reducirt, so dass alle von möglichst der gleichen Höhe waren. Gegen Ende November zeigte sich ein deutlicher Höhen-Unterschied zu Gunsten der Rubidiumpflanzen, der stets zunahm. Die Messung am 17. December ergab :—

Rb- Pflanzen.	Control- Pflanzen.
44.6 cm.	39.1 cm.
46.5 „	46.0 „
46.8 „	47.0 „
54.3 „	49.5 „

Die Höhen-Unterschiede nahmen zu, wie die am 19. Januar anfgenommene photographie (Tafel XXV) gut erkennen lässt. Dabei waren die Rubidiumpflanzen vollständig normal.[2] Wegen Auftretens von Pilzen wurden die Pflanzen schon bald nach der Blütenperiode geschnitten. Das Gewicht betrug :

[1] Das Kaliumcarbonat wurde später separat dem Boden einverleibt.

[2] Ob Buchweizen und andere Pflanzen hiebei ebenfalls normal bleiben, soll noch geprüft werden.

	Rubidiumpflanzen.	Controlpflanzen.
Aehren, Frischgewicht;	6.1 g.	3.7 g.
Lebende Blätter, frisch;	81.3 „	53.3 „
Abgestorbene Blätter, lufttrocken;	5.2 „	4.8 „

Ein dritter Versuch wurde mit *Spinacea oleracea* angestellt. Alle Verhältnisse waren hier die gleichen wie oben bei *Brassica*. Bei den Rubidiumpflanzen erhielt der Boden 50 Milligramm Rubidiumchlorid per Kilo. Als der Samen reif war, wurde geschnitten und die besten Exemplare frisch gewogen.

	Rubidiumpflanzen.	Controlpflanzen.
Gewicht der grössten Pflanze, Varietät I	18.2 g.	12.0 g.
Die zwei grössten Pflanzen der Varietät II	16.5 g.	13.2 g.

Es hatte somit in allen diesen Fällen ein stimulirender Effect des Rubidiumchlorids stattgefunden, was wohl von beträchtlichem theoretischen Interesse ist. Für die Zwecke der Praxis jedoch ist eine Anwendung des Salzes ausgeschlossen, da dessen Preis ein zu hoher ist.[1]

[1] Es kosten 100 g. Rb Cl = 12 Mark (ca. 6 Yen).

On the Stimulating Action of Manganese upon Rice.

BY

M. Nagaoka.

In our last Bulletin the observation was communicated that small doses of manganese administered as sulphate had a very favorable action on the development of various plants. This made it very desirable to carry on a field experiment with rice which is the most important agricultural plant in Japan.

Thirty six wooden frames each representing an area of 0.826 square Meter were placed, three feet apart, into the paddy field of our College farm to a depth of 60 cm., leaving 6 cm. above the ground. The soil had not received any manure the previous three years' and was now manured[1] in the ratio

of 100 Kg. N per ha, as ammonium sulphate
" " " K_2O " " as potassium carbonate
" " " P_2O_5 " " as double superphosphate.

The potassium carbonate was separately applied (June 23) and the other two salts four days later.

On June 29 manganese was applied as manganosulphate in such quantities that the amount of manganic oxid corresponded to the following proportions, three series being observed in each case:—

[1] Before manuring the soil was sifted, and all remnants of former vegetation removed.

No of wooden frames.			Mn_2O_3 per ha. Kg.	Mn_2O_3 per frame. Gram.
1	13	25	0	0
2	14	26	0	0
3	15	27	10	0.833
4	16	28	15	1.250
5	17	29	20	1.666
6	18	30	25	2.083
7	19	31	30	2.499
8	20	32	35	2.916
9	21	33	40	3.332
10	22	34	45	3.749
11	23	35	50	4.165
12	24	36	55	4.582

On July 7 the young rice plants (55 days old) from the seedbed, were transplanted into the frames, each receiving 16 bundles of twelve healthy individuals of equal size.[1] The treatment (irrigaton, etc.) did not differ, from that usually observed with the rice fields in Japan. The weather conditions were not favorable this year for this crop in the whole Empire of Japan, but the relatively low summer temperature diminished on the other hand the dangers from fungi and insect pests with this crop. Our frames remained free from such pests. The crop was harvested on November 29 with the following result, obtained by weighing in the air day condition.

[1] The variety was the *Satsuma*, characterized by its resistance power and medium duration of vegetation.

No. of frames.	Mn_2O_3 per ha. kg.	Full grains. gr.	Empty grains. gr.	Straw. gr.	AVERAGE. Full grains.	Empty grains.	Straw.	Total.
1	no manure	151.6	3.2	193.0				
13	and no	142.6	3.0	171.0	150.3	3.0	185.0	338.3
25	Mn_2O_3	156.5	2.7	191.0				
2	no Mn_2O_3	177.0	4.6	242.0				
14		227.8	6.3	312.8	202.5	5.4	269.6	477.5
26		202.6	5.2	254.0				
3		250.6	6.8	319.6				
15	10	239.6	5.8	285.6	247.3	7.0	308.7	564.0
27		251.8	8.4	321.0				
4		249.9	6.3	401.6				
16	15	257.6	4.8	307.5	256.7	4.4	329.1	590.2
28		262.6	5.1	278.0				
5		277.5	5.3	354.6				
17	20	269.4	7.8	341.6	264.3	6.3	327.7	598.3
29		245.6	5.9	287.0				
6		279.0	10.3	330.6				
18	25	264.5	5.1	335.0	272.1	6.8	348.5	627.4
30		272.7	5.2	326.0				
7		270.0	5.8	374.7				
19	30	256.5	4.4	316.0	267.7	4.2	340.9	612.8
31		276.8	5.3	332.0				
8		264.6	8.2	325.8				
20	35	267.8	4.4	334.0	267.3	6.0	322.9	596.2
32		269.4	5.5	309.0				

No. of frames.	Mn_2O_3 per ha. kg.	Full grains. gr.	Empty grains. gr.	Straw. gr.	AVERAGE. Full grains.	Empty grains.	Straw.	Total.
9		261.8	8.0	364.6				
21	40	269.3	5.6	308.0	272.3	6.9	338.5	617.7
33		285.9	7.0	343.0				
10		256.5	9.0	340.6				
22	45	286.5	7.1	338.0	271.9	7.1	334.9	613.9
34		272.8	5.3	326.0				
11		? (198.6)	(9.0)	312.5				
23	50	270.6	6.7	399.0	278.1	6.6	359.5	645.2
35		287.6	6.5	367.0				
12		254.5	6.7	331.4				
24	55	279.4	5.2	364.1	272.6	4.1	345.2	621.9
36		283.9	6.4	340.0				

The application of manganese had therefore a considerable influence upon the yield, which will be noticed more conveniently by the following table, in which we take the yield in grains of the manured plot without manganese as a unit :—

Mn_3O_4, Kg. per ha.	Harvest of full grains. (Average)
none	1.00
15	1.26
20	1.30
25	1.34
30	1.32
35	1.32
40	1.34
45	1.34
50	1.37
55	1.34

It will be noticed from these figures, that a moderate dose of 25 Kilo Mn_2O_3 per ha led to an increase of the harvest of one third and that higher doses of Mn_2O_3 did not influence essentially this result under the given conditions.

It is further of some interest to examine whether the average ratio between the weight of grain and straw is affected to any extent by the influence of manganese. The quotient of yield[1] $\frac{K. 100}{S}$ which expresses the percentage of grain relatively to straw is for the different cases :—

MANURED PLOTS.

Amount of Mn_2O_3 per ha.	Quotient of Yield.	
No manganese	75	
10 Kg.	80	Average = 79.5
15 „	78	
20 „	82	
25 „	78	
30 „	78	
35 „	82	
40 „	80	
45 „	81	
50 „	77	
55 „	79	

The application of manganese had therefore—*ceteris paribus*—a favorable influence on the quotient of yield.

Let us now determine by calculation whether the application of manganosulfate would be profitable for the farmer. The price of 100 Kilo of pure crystallized manganosulphate is according to the latest pricelist of Theodor Schuchardt = 110 Mark or 53 yen.[2]

The average production *per ha.* of grains of rice with husk is = *3525 Kilo* and of airdry straw = 5250 Kilo.

[1] On the *Quotient of Yield* (Erntequotient) see the article of *O. Loew* in this Bulletin.

[2] 1 Mark = $38\frac{28}{100}$ sen, latest quotation.

The wholesale price of crude rice grains is 9.9 sen per Kg., of airdry straw = 1.2 sen. per Kg. hence the average yield per ha. has a value of 349 yen in grains and 63 yen in straw = 412 yen.

An increase of one third would have an additional value = 137.33 yen while the cost of the mangano-sulphate required would be = 30 yen. Hence the application of this salt on soils poor in manganese would be of advantage. The impure manganous chlorid of commerce would fulfill the same purpose and would cost less than 10 yen in the above case.

On the Physiological Action of Iodine and Fluorine Compounds on Agricultural Plants.

BY

S. Suzuki and K. Aso.

A. On the Influence of Potassium Iodid on Oats.

By S. Suzuki.

I have demonstrated in a former article[1], that potassium iodid in exceedingly high dilution can exert a stimulant action on plant growth. The pea had served for that experiment. I had, however, at the same time commenced an experiment with oats, the result of which are described in the following lines.

Soil and manure were exactly the same as in the former case: each pot contained 2300g. air dry soil and was manured with 3g. Na NO_3, 3g. K_2CO_3 and 4. 6g. common superphosphate. The seeds were sown (15 in each pot) on Feb. 21 and the young shoots reduced on March 7, to five per pot of equal height. Pot No. I. received on March 11 and 25, April 14, 21 and 28 and May 6, each time 0.01g. potassium iodid dissolved in 100 c.c. water; further pot No. II. 0.001g. and No. III. 0.0001g. of that salt, while No. IV. served as control. Those quantities of potassium iodid expressed in percentage of soil are:

No. I. = 0.00 2609 %
No. II. = 0.000 2609 %
No. III. = 0.00002609 %

[1] Bull. College of Agriculture, Toyko Vol. 5. No. 2. p. 199.

In the beginning of May, the tips of the leaves of No. I. turned reddish yellow and further growth was retarded, but an increase of shoots made up for the loss in height, leading finally to an increase in the yield compared with the control plants (compare the photograph, Plate XXVI). The plants were irrigated almost daily with 300 c.c water until the flowering stage was reached, after that with 500 c.c. The flowering period was over on May 16. The plants were cut on July 6. The straw and the grains, unhusked, were weighed in the air dry state with the following result:

	I.	II.	III.	IV.
Number of Stalks.	15	14	14	9
Weight of grains, unhusked, g.	24.8	25.5	27.2	21.4
Weight of straw, g.	48.5	56.6	58.4	45.2

The result undoubtedly proves a stimulant action of iodine, even if present in such a small quantity as 2. 6g KJ in 10000 Kilogram of soil as in No. III. The increase however, is with oats not so large as with the pea (These Bulletins, vol. V p. 199).

I had mentioned already in my former article, the experiment of *A. Vœleker* who soaked seeds of wheat and barley for a short time in a 1% solution of sodium iodid and observed with such seeds, an increase of yield. The quantity of sodium iodid that penetrated into those seeds must then have been exceedingly minute, otherwise a poisonous effect would have shown itself. I have repeated that experiment with oats. The seeds were soaked for 24 hours in a 1% potassium iodid solution, washed and then sown in two pots, 15 seeds in each. Later on the young shoots were reduced to five of equal height. After a few weeks, it became clear that the plants did not so well develop as the control plants. This may be due to more iodid having entered into the grains than in the case described by Vœleker. This difference is probably caused by the prolonged soaking in my case. Also differences of temperature during the soaking process can influence the result. The plants were cut on July 13, and the straw and grains, unhusked, weighed in the air dry state with the following result:

	I.	II.	Control.
Weight of grains, unhusked.	18.1 g.	16.8 g.	21.4 g.
Weight of straw,	24.4 „	22.5 „	45.2 „

This shows that the amount of KI absorbed in the soaking was large enough as to cause a retarding influence, which was much greater, however, in regard to the production of straw than in that of grains.

A field experiment further was made with oats. On 3 plots, each measuring 20 square meters, an equal amount of oats grains previously soaked for two days in water was sown on March 21. On April 15, the young plants had reached 3—4 cm. and were treated now the first time with potassium iodid solution[1]. The treatment was repeated on Apr. 22, May 7 and 22, and June 10. The total quantity of potassium iodid applied to the plot No. I. was 0.25g., to the plot No. II. 0.025g. On June 26, flowering commenced, on July 16, some spots of rust became visible. On August 6, the plants were cut, but owing to several storms, some loss of grains had occurred; hence the final weight is somewhat below the actual production. The straw and grains, unhusked, were weighed in the air dry state with the following result:

	I.	II.	Control.
Weight of straw and grains.	6.96 Kg.	6.08 Kg.	6.05 Kg.
Weight of grains.	0.99 „	0.78 „	0.83 „

A small increase of yield had therefore taken place by the application of 0.25 g. KI for 20 □ Meters, while 0.025g had no influence.

[1] The solution was highly diluted, each dose of potassium iodid being dissolved in 10 litres of water.

B. On the influence of potassium iodid on radish

By S. Suzuki.

The same plots[1] which had served for the culture of oats just mentioned served for this experiment with radish. One plot received 0.5 g. potassium iodid in one dose that is double the quantity of that of the last experiment wish oats, the next plot received 0.05 g. potassium iodid in one dose (also double of the last experiment). The radish seeds were sown Oct. 1 and the young plants were thinned out on Nov. 4. After four weeks a considerable difference in favor of the iodine plants was noticed. On each plot (20 square meters) were grown 60 plants, which were harvested on Dec. 24. The results are as follows:—

		0.5 KJ.	0.05 KJ.	Control.
Large plants. Average periphery of roots = 9.5 c.m.	Number.	19	23	10
	Weight.	5440 g.	7500 g.	2770 g.
	Weight of roots.	2370 „	3360 „	1440 „
Middle sized plants. Average periphery of roots = 7.5 c.m.	Number.	24	20	15
	Weight.	4020 g.	4220 g.	3020 g.
	Weight of roots.	1370 „	1540 „	1010 „
Small plants. Periphery of roots = 4.7 c.m. and less.	Number.	17	17	35
	Weight.	1960 g.	1980 g.	3110 g.
	Weight of roots.	520 „	510 „	790 „
Total weight of plants.		11420 g.	13700 g.	8900 g.
„ „ „ root.		4260 g.	5410 g.	3240 g.

[1] Each plot was manured with 200 g. double superphosphate, 312.5 g. $(NH_4)_2 SO_4$, and 312.5 g. wood ash, the latter being given in a highly diluted state ten days later.

This result shows a very favorable influence of potassium iodid in small quantities on the yield with radish. A calculation as to the outlay and profit is of some interest.

KI applied for 20 square meter	= 0.05 g.
Corresponding for 1 ha	= 25 g.
its value	= 0.6 yen
The increase in harvest per 20 sq.m.	= 2170 g. root,
Corresponding per ha	= 1085000 g. „
	= 289 Kwamme.
its value	= 2.89 yen

Hence it would certainly be profitable to apply small doses of potassium iodid to the field; the costs would be however very trifling, if we would substitute the crude ash of seaweeds for the purified potassium iodid[1]. It might be here also called attention to the interesting fact that the farmers along the coast of Japan apply sea weeds as a green manure with very much success, which very probably is not only due to the small quantities of potassa, nitrogen and phosphoric acid, but also to some extent to the small doses of iodine present. Finally I might point out that it might not be advisable to make an application of iodine compounds every year on the same field, since the iodine might gradually be increased to a point where the stimulating action ceases and a noxious action commences. An application on only every second or third year might therefore be preferable.

C. On the influence of sodium fluorid on oats.

By K. Aso.

In a former article[2] was shown that fluorine in the form of sodium fluorid applied in exceedingly high dilution on barley, wheat, rice, soy-bean

[1] Since this ash contains about 5 per mille iodine, 5 Kilo of it would suffice to supply the necessary quantity per ha.

[2] Bul. College of Agriculture, Tokyo, Vol. 5. No. 2. p. 181.

and pea plants, can exert a stimulant action.[2] In the following lines another experiment with oats will be described. All the conditions in regard to soil, manuring, time of sowing, kind of seed, the number of shoots, watering and harvesting were exactly the same as in the above described pot-experiment made with potassium iodid *by S. Suzuki*; hence the reader, is referred in this regard to the introductory remarks of the above communication.

Pot No. I received on five days 0.01g. sodium fluorid in 100.c.c. water, No. II. 0.001g and No. III. 0.0001g, while No. IV served as control. The applications of the highly diluted solutions of sodium fluorid were made on March 11, April 14, 21 and 28, and May 6. On May 20, it was noticed that the plants of No. II. developed best, then followed those of No. I. There was hardly noticed any difference between the plants of No. III, and No. IV. The color of the leaves of No. I. was a little paler than that of the control plants. On May 29, the number of ears was:

No.	I.	3
No.	II.	4
No.	III.	4
No.	IV. (control).	2

The plants were cut on July 6. The straw and grains, unhusked, were weighed in the air dry state with the following result:

	I.	II.	III.	IV.
Number of stalks,	8	9	10	9
Weight of grains, unhusked, g.,	23.0	24.2	25.5	21.4
Weight of straw, g.	50.1	45.6	48.6	45.2

This result undoubtedly shows a stimulant action of fluorine in the proportion of 2.17g. in 10000 kilo soil as in No III, although the differences are here not so large as in the case of the pea, described in a former Bulletin.

[2] Although the presence of fluorine has to be assumed almost in every soil, it is of special interest that it occurs naturally in wines from certain countries (Holzman).

[1] Cf. Bul. V. No. 2.

D. On the influence of sodium fluorid on radish.

By K. Aso.

Two plots, each measuring 10 square metre, had received during the summer, 0.6g and 0.06g NaF., and again shortly before sowing the seeds of radish, they received 0.8g. and 0.08g. sodium fluorid respectively. The manure was the same as mentioned in the above field experiment with potassium iodid. The radish was sown on October 1, and the young plants thinned out on Nov. 4. Towards middle of December a difference in development between these plants and control plants was very plain. The plants were harvested on Dec. 24 with the following results:—

	a. (0.14 g. NaF)	b. (1.4 g. NaF)	control.
Total weight	7970g.	6490g.	3814g.
Weight of the ten largest roots,	2050g.	1470g.	617g.

A stimulating action of considerable magnitude is therefore quite evident and it is of special interest that the smaller quantity 0.14g. sodium fluorid has produced a better result than the ten times larger quantity. The cost of production of the increased amount of radish is to be seen from the following calculation:

NaF applied for 10 square metres	= 0.14 g.
„ corresponding to 1 ha.	= 140 g.
Its cost	= 8.4 sen.
Increase of harvest per 10 sq.m.	= 4.156 Kg.
„ corresponding to 1 ha.	= 4156 Kg.
Its value	= 110.6 yen.

On the Chemical Nature of the Oxidases.

BY

K. Aso.

The oxidases are considered generally as kinds of enzyms and indeed various of their properties are in favor of this view. Also the observation of *Slowtzoff*[1] and of *Epstein*[2] seem to fully establish the enzym-nature of the oxidases. Recently, however, *J. H. Kastle* and *A. S. Loevenhart*[3] described experiments which seem to indicate a certain analogy between the behavior of oxidases and that of organic peroxids towards certain antiseptics and poisons. These authors conclude therefore: "the oxidizing ferments are peroxids, formed when autoxidable substances come in contact with air and these peroxids give up a part of their oxygen to other less-oxidizable substances present in the cell." "In other words, that the process of rendering oxygen active by the living cell, is probably brought about in essentially the same way that this is accomplished by phosphorus, benzaldehyd and other oxygen carriers, viz, as one phase of autoxidation." Further, these authors hold that "the function of hydrogen peroxid in the guaiacum hydrogen peroxid reaction, is to react with some one or more of the organic substances present in the plant or animal extract to form an organic peroxid."

[1] Z. Physiol. Chem. 31. *Slowtzoff* observed that the action of laccase is proportional to the square-root of its quantity. He considers the laccase as a peculiar protein substance which is not changed by pepsin or pancreatin. In the pure state, it is killed already at 50°c, in presence of mineral substance however between 65-70°c.

[2] Arch. Hyg. 36. p. 140. *Epstein* observed that the presence of hydrocyanic acid in small quantities prevents the action of oxidase and that after the removal of hydrocyanic acid, the activity of oxidase is restored.

[3] Americ. Chem. Journ. XXXVI. No. 6. Dec. 1901.

These authors based their view principally on the following special observations:

1. Benzoyl-phthalyl-and succinyl peroxids give directly guaiacum blue with tincture of guaiac. Hydrogen peroxid alone gives a faint blue color and that only on heating with guaiacum tincture. The authors mentioned further, that lead dioxid and manganese dioxid give the blue color. But from these facts certainly does not follow that every substance which can produce a blue color with guaiacum must be of the nature of a peroxid. Indeed lead dioxid and manganese dioxid are no genuine peroxids like barium peroxid is and further we find that not only every weak oxidizing agency (nitrous acid, ferric chlorid, potassium ferricyanid), but also oxid of silver and further quinone may produce the blue guaiacum reaction at once.[1] Some other observations of these authors are however of considerable interest, namely the bluing of the guaiacum tincture by benzoylperoxid can be prevented by hydroxylamine, and phenylhydrazine. In the same way, also the oxidizing action of the potato juice on guaiaconic acid can be inhibited. However, it might be objected that hydroxylamine and phenylhydrazine might merely destroy the guaiacum blue, while they do not counteract the oxidizing activity itself. Indeed, I have already observed some time ago that the blue color produced with guaiacum tincture by the action of oxidase disappears, when a little free hydroxylamine is added. Also phenylhydrazine (0.5%) decolorized the freshly formed guaiacum blue. The inhibitive effect of hydrocyanic acid on the action of oxidase was observed again by *Kastle* and *Loevenhart*[2] after *Epstein* and also myself had made the same observations.

A further observation of those authors relates to the inhibiting action of sodium thiosulfate. When to 2c.c. of a potato extract 0.5c.c. of $\frac{N}{100}$ solution of the thiosulfate was added, the blue color with guaiacum was prevented. Since other enzyms are not injured by sodium thiosulfate, it was inferred that the oxidase is no enzym proper. But here it might be objected

[1] Quinone produces a blue color also with the tetra paper of Wurster. The common quinone is probably a diketon and not a peroxid as formerly believed (Fittig).

[2] They observed that "9 parts prussic acid in 10 million parts of juice very nearly mark the limit of the poisonous effect of that acid on the oxidizing substances in the potato.

that an oxidizing enzym must naturally contain differently constituted active atomic groups than the other merely hydrolyzing enzyms, devoid of any oxidizing power;[1] further it might be objected that the oxidase caused the oxidation of thiosulfate sooner than that of guaiaconic acid.

I had observed a year since that the guaiacum blue may easily be decolorized by certain compounds present in some plant juices as, e.g., in that of radish and it requires often a certain excess of guaiacum tincture to preserve the blue guaiacum reaction for some time. Also tannin not only prevents the usual guaiac reaction of peroxidase, but in certain quantities can also bleach out again the blue color after it has made its appearance.[2] Such facts might also apply to the observation of *Kastle* and *Loevenhart* that the onion bulb gives no guaiac blue reaction. I can confirm this statement, but if we precipitate the enzyms of the onion with alcohol first and thus remove compounds that interfer (allylsulfid?), the guaiacum reactions for oxidase and peroxidase, can be obtained with the aqueous solutions of these enzyms, although these reactions set in here more slowly than in other cases. I might further add that the onion juice shows an unusually strong acid reaction and that after neutralization also the guaiacum blue reaction can be slowly produced with the juice itself.

Recently Bach and Chodat observed that the juice of *Lathraea squamaria* yielded on addition of some diluted baryta water, a precipitate which after treating with dilute sulphuric acid produced at once an intense blue color on paper impregnated with starch paste containing potassium iodid.[3] Their conclusion is, "Die sofortige Jodausscheidung aus Jodkalium konnte daher nur von einem acylirten Hydroperoxid herrühren."

[1] A similar observation was made by the writer in regard to the behavior of enzyms toward sodium fluorid; while most enzyms are not injured by it, the oxidase proper (laccase) is killed easily.

[2] Compare my article, "On the Rôle of Oxidase in the preparation of Commercial Tea; Bul. College of Agric. Tokyo, Vol. IV, No. 4. p. 256.

[3] Schönbein had observed as early as 1864 (Journ. für prakt. Chem. Bd. 88.3.460) that certain aqueous extracts of plants give a blue reaction with acidulated iodid of potassium starch, which reaction he supposed to be due to nitrous acid. Many plant juices however yielded that reaction only after standing for a series of days. In the latter case, nitrite might have been produced from the nitrates, frequently present in plants, by bacterial action.

But we must take into consideration that iodine can be very easily liberated from potassium iodid by the most different oxidizing influences, in presence of an acid reaction. These authors also observed that some plant juices will lose the property of liberating iodine within a few minutes. If this is so, we have already a clear proof before us that this oxidizing principle is not identic with the oxidase characterized by the guaiacum blue reaction, since this can still be easily observed in a plantjuice after a few days, although the reaction will then be weaker. Also the further interesting observation of these authors that in the wilting of a plant the iodine reaction disappears first, militates against the identity of this oxidizing principle with the common oxidase (laccase).

I have made a series of tests with the juices of potato tubers and the root of radish, which yield the guaiacum reactions for oxidase and peroxidase very well. But, with these juices, I could not observe the iodine reaction. As I supposed that these juices might contain some substance which interfered with the formation of iodine starch, or absorb the iodine immediately after being liberated I have treated those juices with an excess of absolute alcohol and after washing the precipitates, containning the oxidizing enzyms, with alcohol they were dissolved again in some water. These solutions also yielded the guaiacum reactions upon oxidase and peroxidase very well, but not a trace of the iodine reaction. I applied for one volume of this solution $\frac{1}{3}-\frac{1}{2}$ volume of a 2% starch paste to which 1% potassium iodid and 0.5 % acetic acid was added. These mixtures yielded even after twenty four hours standing in darkness, no trace of any blue reaction, while the guaiacum blue reaction even in absence of hydrogen peroxid was still obtained with great intensity.[1]

Bach and *Chodat* recommend to add some mangano-sulfate in those cases in which the iodine reaction with plant juices fails. But in the above mentioned cases with the juices of potato and radish, this sulfate did not

[1] In one case I had applied intentionally a potassium iodid solution not freshly prepared, but one which had been exposed in presence of air for a few days to sunlight. In this case, a blue reaction was gradually observed, evidently due to slight traces of free iodine formed in this solution.

change the result. However after such mixtures were left for a series of hours to themselves a weak reaction set in. But also in some control tests without plant juices, I observed that mangano-sulfate alone in presence of some acetic acid can gradually cause the liberation of some iodine.

In order to decide whether the oxidizing enzyms are really organic peroxids, I have made the following experiments relating to the special oxidizing enzym, which produces a red color with a 1% guaiacol solution of weak acid reaction. The juice of the leaves of radish contains besides oxidase and common peroxidase, also a peculiar oxidizing enzym which produces the red reaction just mentioned.[1] This juice was mixed with $\frac{1}{10}$ of its volume of a hydrogen peroxid of about 2% and of a faint acid reaction. After five minutes standing, about four times the bulk of absolute alcohol was added and the precipitate very well washed with alcohol This precipitate was then dissolved in some water and tested with guaiacol. but *no reaction whatever was taking place*. If *Kastle* and *Loevenhart's* view was correct, then the supposed organic peroxid would be formed almost instantaneously when hydrogen peroxid comes in contact with the proper organic material in the juice. This supposed organic peroxid would consequently be also present in the alcoholic precipitate containing all the oxidizing enzyms, hence the aqueous solution of this precipitate ought to give now without the further aid of hydrogen peroxid, the red guaiacol reaction, but the fact was: *no reaction in absence*, but an intense reaction in presence of hydrogen peroxid. What is true for this kind of peroxidase (β-guaiacolase) is very probably also true for the common peroxidase characterized by the blue coloration with guaiacum tincture and hydrogen-peroxid,[2] but thus far I was not able to prove it in the way just mentioned.

[1] Since *Bourquelot* observed in the fungus *Russula*, an oxidizing enzym which produces a red color with guaiacol even in absence of hydrogen peroxid, I propose to distinguish this peculiar enzym as α-guaiacolase, from the above-mentioned enzym which I call β-guaiacolase. About this reaction compare also my article, 'On Oxidizing Enzyms in the Vegetable Body' Bul. College of Agric. Tokyo, Vol. V, No. 2. p. 207—235.

[2] On heating the solution of the enzym precipitate above mentioned for 5 minutes to 75°, the oxidase and the common peroxidase are killed, while the guaiacol-hydrogenperoxid reaction was still obtained although weaker.

since I encountered some difficulty in the preparation of a peroxidase precipitate snfficiently pure. It cannot be denied that a transient formation of an organic peroxid takes place when the oxidase causes the oxidation of a certain other compound. *Such peroxids are then the first products of an oxidation caused by the oxidizing enzym and this opinion seems to be also that of Bach and Chodat, and differs essentially from the hypothesis of Kastle and Loevenhart, according to which oxidases themselves are the peroxids.*

It must be remembered, moreover, that the liberation of iodine from potassium iodid not only may be due to different oxidizing influences but also that on the other hand, it is not a specific property of all organic peroxids. Thus neither diethylperoxid nor dibenzoylperoxids will liberate iodine, but *benzoylhydroperoxid can do so.* But it is a very striking fact that this peroxid can also liberate iodine from potassium iodid in the presence of sodium bicarbonate and not only in presence of free acid. Such *hydroperoxids* as can liberate iodine are exceedingly powerful compounds,[1] resembling hypochlorites in their actions ;[2] hence the amount of such poisons the cells can only be exceedingly minute.

Since I have now proved that the iodine reaction does not go parallel to the blue guaiac reaction and since further there exists no proof that organic peroxids are the cause of the iodine reaction in many vegetable objects, it was important to decide the nature of the iodine liberating substance. Two suppositions seemed to deserve some consideration, either there might exist certain organic ferric compounds in some objects or traces of nitrites. The following lines will doubtless prove of some interest in regard to this question.

[1] Recently, *E. H. Fage* (Amer. Pat. 717016 of 30. Dec. 1902) described acetylhydroperoxid $CH_3-C(=O)-O-O-H$ which has a strong odor after hyprochlorous acid and has a powerful bactericida action.

[2] Compare in regard to the data here mentioned, the articles of *Bayer* and *Villiger* in Berichtel der Deutschen Chemischen Gesellschaft, 1899 and 1900, especially in the latter volume, page 1578.

Experiments with Buds.

Sections of potato-buds yielded directly the iodine reaction in presence of some acetic acid.[1] Also the blue guaiac reaction was directly produced. The cold prepared extract behaved alike. In a second case, with buds from other potatoes, however, the iodine reaction failed, although oxidase, peroxidase and β-guaiacolase[2] were present. Of considerable interest were the observations made with the tubers and buds of *Sagittaria sagittæfolia.*

The cold prepared aqueous extract of the bulb gave no iodine reaction, but it gave the blue guaiac reaction, while the extract of the buds yielded directly both reactions. Eight buds of *Sagittaria* were extracted with 100c.c. water; a portion was tested directly and another after boiling for $^1/_2$ minute. In the former case, oxidase, peroxidase and β-guaiacolase were easily recognized by their reactions, while in the latter case, no trace of this reaction was more obtained. But very different were the phenomena in regard to the iodine reaction. *Not only the unboiled, but also the boiled juice yielded this reaction with great intensity after addition of some acetic acid.*[3] Even boiling for 2 minutes did not alter this result.[4] It is therefore undeniable that hereby another proof is furnished that the substance which gives the guaiac reaction for oxidase is not identic with the substance that give the iodine reaction. It was of interest to me to decide whether in the *bulbs* a compound would be present that can prevent the iodine reaction which so easily and intensely is obtained in the *buds.* Hence four bulbs were crushed after removing the skins and macerated with 100c.c. water. The filtrate was mixed with alcohol, whereby a considerable precipitate

[1] The tubers did not give this reaction, as was mentioned above.

[2] See above p. 485.

[3] A blind control test with acetic acid and potassium iodid-strach paste showed no reaction whatever.

[4] *Bach* and *Chodat* mention that heating to 80°C prevents the iodine reaction. This is, however, probably only the case when the acidity of the juice is more marked than in the case of the *Sagittaria* buds. It can then be very easily explained that ni rous acid set free reacts upon amido-compounds and is destroyed with development of nitrogen.

was obtained. This was filtered off, the residue washed and after well pressing between filter paper and evaporation of the alcohol at common temperature, extracted with water and tested again. The iodine reaction failed however while the guaiac reaction was obtained. Further tests convinced me that among other substances soluble albumin as well as pepton can prevent the appearance of the iodine reaction which very easily can be understood, since these compounds can bind some iodine, thus rendering formation of iodine starch imposible. Since, the juice of the bulb contained some soluble albumin, it was not surprising to find that the juice of the *bulb* was capable to prevent the iodine reaction with the juice of the *bud*, and further that the *boiled* juice of the bulb did not prevent any more that iodine reaction with the juice of the bud.

It was further tested whether the juice of the bulb itself would yield the iodine reaction after removing the soluble albumin. But after a few seconds boiling whereby the albumen separated in flocculi, no iodine reaction was obtained in the filtrate, although short boiling does not destroy the active compound as I have mentioned above.

The resistance of the active principle towards boiling heat suggested to make a careful test for nitrites and indeed to my great surprise the reaction of *Griess* for nitrites yielded at once a very decisive result. Hence the liberation of iodine is due not to any enzym nor to any peroxid, but to nitrites.

It is very strange that the occurence of nitrite in plants thus far was overlooked. It is true that *Schönbein* more than thirty years ago had supposed the existence of nitrite in plant juices and further that *Berthelot* had assumed the formation of nitrate in leaves and shoots from ammonia. But some authors did not agree with these observations. The occurrence of nitrite in plants is indeed surprising, since we know that nitrites are very poisonous for plants with an acid plant juice.[1] But in this regard we must not overlook that the quantity of nitrite present in these shoots is only very small, and that nitrous acid can here not exist in the free state since the acidity of these shoots is exceedingly weak.

[1] O. Loew, Natürliches System der Giftwirkungen, p. 61 and p. 109.

Since the question is of some interest whether this nitrite is formed by reduction of nitrate or by oxidation of ammonium salts, I have tested the bulbs with diphenylamine, but no reaction was obtained. The boiled juice of the buds was also poured carefully on the surface of diphenylamine solution in concentrated sulphuric acid, and here *soon observed a blue ring*, probably due to the small quantity of nitrites present.[1] A strong reaction for nitrites could not be expected in this manner, since we know that nitrites are in presence of some strong acids and amido-compounds very quickly destroyed with evolution of nitrogen. We can therefore infer that nitrous acid in the buds in analogy to nitrification process is formed by oxidation of ammonia.

Summary.

It is very improbable that the oxidase and peroxidase of plant juices are organic peroxids. The liberation of iodine by plant juices was proved in one case to be due to traces of nitrite and it is probable that these are present sometimes also in other plant juices. The iodine and the guaiac reactions do not show any parallelism.

[1] The reaction of Griess sometimes may be prevented by the presence of certain benzene compounds, like tannins.

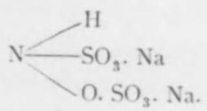
Can Sulfo-derivatives of Hydroxylamine Serve as a Source of Nitrogen for Plants?

BY

S. Suzuki.

It is well known that hydroxylamine as well as diamidogen are not only incapable of furnishing nitrogen to phænogams but they are directly poisonous.[1] But thus far no tests have been made with the sulfoderivatives of these compounds. It seemed to me of some interest to test one of such derivatives in this line. I selected the sodiumsalt of α—β hydroxylamine-disulfonic acid which preparation was kindly furnished me by Prof. T. Haga.[2]

This compound has the formula.



I prepared the following solutions:—

a) 1 per mille Calcium acetate.
1 „ „ Magnesium sulfate (anhyd.)
1 „ „ Potassium chlorid.
1 „ „ Ferrous sulfate (anhyd.)

b) 2 per mille Dipotassiumphosphate.
1 „ „ Sodium α - β. disulfhydroxylamate.

and for the control plants:

c) same as a).

[1] Loew, Ein natürliches System der Giftwirkungen, 1893, p. 41.

[2] This interesting salt was prepared by Prof. Haga from oximido-sulphonate of sodium by a complicated process which will be published later by that author.

d) 2 per mille Dipotassium phosphate.
0.28 „ Ammonium sulfate.[1]

The application of two different solutions instead of a single one containing all mineral nutrients was necessary for the following reason. The above named derivative contains two sulpho-groups which on being set free by a decomposition would probably form an acid salt which in itself would be injurious. Hence I applied the secondary potassium phosphate in place of the usually applied monopotassium phosphate. But since that phosphate would precipitate the lime and magnesia of the nourishing solution, it had to be applied separately together with the above named derivative. Barley shoots (about 13 c.m. high) were placed on Nov. 21. in the solutions a) and c), on the following day in the solutions b) and d). This manipulation was repeated in this way for nearly 7 weaks. Two series with two shoots in each flask were observed. On Dec. 15 the plants were transferred to larger flasks and all solutions renewed. At this time it was evident, that the control plants showed a much better development. From then to Jan. 10th, a decided starvation was noticeable; all the old leaves died off, while young leaves developed but remained of very small size. The experiment was closed on Jan. 10th, since there was no further growth observed and only very few leaves had remained green, while in the control case the plants looked vigorous and healthy. It seems further that the plants can not regenerate hydroxylamine to any noticeable degree from the salt, since a very poisonous action would have soon become noticeable. The state of affairs at the close of experiment is seen in the following table:—

	Number of stalks.	Number of leaves. living	dead	Length of the longest leaves.	Weight of the fresh plants.
Solution of a and b series.					
A 1	1	3	6	13 c.m.	2.68 g.
A 2	1	3	6	11 „	
B 1	1	2	6	12 „	2.00 „
B 2	1	2	5	11 „	

[1] This ammount of ammonium sulfate corresponds to that of the sodium salt of α β. hydroxylamine disulfonic acid, in the solution (b), in regard to the amount of nitrogen.

Control plants.

C 1	5	12	5	17.5 c.m.	7.02 g.
C 2	4	7	4	17.5 „	
D 1	8	13	4	17.5 „	8.42 „
D 2	4	9	4	16.5 „	

Experiment with fungi.

A culture solution was prepared, consisting of

200 c.c. Water.
2 g. Canesugar.
0.4 „ Hydroxylamine disulfonate of sodium.
0.2 „ KH_2PO_4.
0.01 „ $MgSO_4$.

Two flasks, a and b, each containing 100 c.c. of this solution were after sterilisation infected with:

a) *Penicillium glaucum.*
b) *Bac. methylicus.*

An infection in bouillon from the same source served as control. After 14 days there was no trace of development noticed in the main flasks, while the control flasks showed luxuriant growth.

My general conclusions are therefore:—

1. The α. β. hydroxylamine disulphonic acid is no direct poison for the barley but it is incapable to furnish nitrogen and hence the plants undergo starvation when nitrogen is supplied in this form.[1]

2. Development of fungi is impossible, when in the culture solutions the nitrogen is offered in the form of α-β-hydroxylamine disulfonic acid.

[1] It is of some interest to compare with this result the poisonous character of amido-sulfonic acid, these Bulletins, vol. II, page 487 (1897).

On the Influence of a Certain Ratio Between Lime and Magnesia on the Growth of the Mulberry-tree.

BY

K. Aso.

Since sericulture is one of the most important agricultural industries in Japan, much attention is paid to the cultivation of the mulberry-tree, and various investigations have been published in relation to it. A short review of some of these may be not out of place.

The composition of the ashes of healthy mulberry-leaves was found in average, as follows:[1]

P_2O_5	12.02%
K_2O	31.47%
Na_2O	3.14%
CaO	33.15%
MgO	12.48%
SO_3	4.64%
Cl	0.06%
SiO_2	1.45%
Fe_2O_3	1.59%

On analysis of the bark of the healthy mulberry-roots (var. Nezumigaeshi) collected on Dec. 4., I have obtained the following result:

In 100 parts of the crude ash,

P_2O_5	18.48
K_2O	9.39
CaO	35.50
MgO	7.34

[1] Nagaoka: Chemical Tables for Daily Use, p. 84.

SO_3	1.38
SiO_2	2.40
Fe_2O_3	8.73

U. Suzuki[1] has determined the lime and magnesia content of healthy and dwarf-diseased leaves without however observing great differences; the diseased leaves contained, like the healthy, from 2 to 4 times as much lime as magnesia, although in most cases, the ratio between these was somewhat greater in healthy than in diseased leaves.

Maeno[2] observed in mulberry-leaves after liming the soil, a moderate decrease of the percentage of woody fibre and increase of the non-nitrogenous extract; further by applying lime, sodium nitrate and calcium sulfate, not only some increase of the non-nitrogenous extract, but also of the protein and fat.

Since the so-called dwarf-disease (Schrumpf-Krankheit) causes an immense damage to the mulberry plantations in Japan, it seemed to me of interest to look also into the composition of such soils as seemed especially favorable for the development of the disease, that is, causing such a condition of the plant as would render it more susceptible for that disease. I restricted myself to the determination of those quantities of lime and magnesia which are available to the root, and for this purpose I have treated the soil with cold hydrochloric acid (10%) for 48 hours. Our experiments with other plants had sufficiently shown that the ratio between lime and magnesia in the soil has a most powerful influence on the development. My analyses, indeed, have shown that the amount of magnesia predominated over that of lime, which is a very unfavorable condition.

In 100 parts of dry soil,

LOCALITY.	Ca O	Mg O
Ōtakaramura, (Aichiken)	0.232	0.332
Jōtan Sericultural School, (Kyōtofu)	0.115	0.259
Angamura (Kyōtofu)	0.150	0.388

[1] Bul. College of Agriculture, Vol. IV. No. 3.

[2] Ibid. Vol. II. No. 7. p. 495.

The following experiments will prove, indeed, that a normal and good development of this plant depends to a great extent on the ratio of lime and magnesia offered to the roots.

Experiment with Water Culture.

Three young mulberry plants (var. Takasuke) with stems about 15 cm. high were placed June 9, in glass vessels of 3 litres capacity containing the following solutions:—

	I.	II.	III.
$Ca(NO_3)_2$	0.5%	0.3%	0.1%
$Mg(NO_3)_2$	0.1%	0.3%	0.5%
KHP_2O_4	0.1%	0.1%	0.1%
$(NH_4)_2SO_4$	0.1%	0.1%	0.1%
$FeSO_4$	trace	trace	trace

On July 11, there was not yet any other difference observed except in the number of rootlets that had developed. There were very numerous rootlets in I and II, while none in III.

On July 25, it could be clearly noticed that in solution III, the leaves developed were very small and of pale green color. On August 8, these small leaves had withered while those developed in the other two solutions appeared healthy and of dark green color, but it was further noticeable that the leaves in I were darker green and smaller than those in solution II. Further, while no rootlet were developed in solution III, numerous rootlets had appeared in I and II. This experiment shows that an excess of magnesia over lime is very injurious for the mulberry tree.

Experiment with Soil Culture.

Each pot contained about 3.8 K. dry soil of an unmanured field from Nishigahara, Tokyo. The content, in the fine earth, of lime and magnesia soluble in hot concentrated hydrochloric acid was as follows:—

In 100 parts of dry soil,

Ca O 1.5
Mg O 1.8

As the development of mulberry-roots is very vigorous, it could be assumed that these amounts of lime and magnesia might be assimilable for this tree. I altered the ratio between lime and magnesia in this soil by mixing calcium carbonate or magnesium carbonate with it to reach the following ratios:—

Pots	Ca O	:	Mg O	
a	1	:	3	
b	1	:	2	
c	1	:	1	(original)
d	2	:	1	
e	3	:	1	
f	4	:	1	

The surface of the pots measured 0.0495□m. As general manure served:

7.5 g. N in the form of ammonium sulfate and
5.6 g. P_2O_5 in the form of potassium phosphate for each pot.

These salts were applied in solution. Young mulberry plants (var. Shihōzaki) of equal size, weighing 976.7 g.—1014.3 g. and of stem-length of about 30 cm. were planted on April 21.

On June 6, and Sept. 19, the following observation was made:—

Date.	I.	II.	III.	IV.	V	VI.
June 6.	Leaves very small.	Leaves small.	Control.	Developed best.		
Sept. 19.	One plant died. With another the leaves are very small.	Leaves developed to some extent.		"	Developed very well nearly, same as IV.	Less well developed than V.

On Oct. 1. A photograph was taken (plate XXVII) which exhibits the great difference of development at once. On Oct. 2 the following observations were made:—

No. of pot.	$\frac{Ca O}{Mg O}$	Number of leaves.	Fresh weight of total leaves, g.	Average weight of one leaf.	Number of branches.	Remarks.
I.	0.33	8	1.5	0.19	3	Branches were very small.
II.	0.5	16	6.8	0.41	4	
III.	1	21	9.8	0.45	7	
IV.	2	30	31.9	1.05	7	One branch was longest of all.
V.	3	38	36.4	0.98	8	Average development of branches was here better than in IV.
VI.	4	20	13.5	0.68	5	

Taking now in consideration, that the plant with the lime factor 2 had the longest branch all that other branches were smaller than with the lime factor 3, which latter had also one branch more, it will be safest to conclude that the best ratio Ca O : Mg O for the mulberry tree lies between 2 and 3. If follows further that an excess of magnesia over lime depresses the growth considerably; the leaves become smaller, but true symptons of dwarf-disease are not observed.

On the Influence of Different Ratios between Lime and Magnesia upon the Development of Phaseolus.

BY

G. Daikuhara.

The knowledge of the physiological functions of lime and magnesia is not only of theoretical but also of practical value, as shown by the recent publications of *Loew*, *May*, *Aso* and *Furuta*. *O. Loew* has named the ratio of $\frac{Ca\ O}{Mg\ O}$ most favorable for plant development the lime factor, taking the absolute quantity of the available magnesia as the unit. Thus it was found that the lime factor for buckwheat is 3, for cabbage 2, for oats 1.

I have sought to determine this limefactor for Phaseolus and also to observe whether an increase of the absolute quantities of those bases would have any modifying influence upon the result.

Thirty small zinkpots of about two Liters capacity served for this experiment. Each received 2,5 Kilo pure quartzsand, mixed with the carbonates of lime and magnesia in the following quantities and ratios:—

Total quantities of $Ca\ CO_3 + Mg\ CO_3$ for the air dry sand:		A. 0.05%	B. 0.1%	C. 0.2%
Limefactor: $\frac{Ca\ O}{Mg\ O}$	I	$\frac{3}{1}$	$\frac{3}{1}$	$\frac{3}{1}$
	II	$\frac{2}{1}$	$\frac{2}{1}$	$\frac{2}{1}$
	III	$\frac{1}{1}$	$\frac{1}{1}$	$\frac{1}{1}$
	IV	$\frac{0.5}{1}$	$\frac{0.5}{1}$	$\frac{0.5}{1}$
	V	$\frac{0.33}{1}$	$\frac{0.33}{1}$	$\frac{0.33}{1}$

As general manure for each pot served :—

$K_2H\,PO_4$ 0.1%

$K\,H_2PO_4$ 0.1%

KNO_3 ..... 0.2%

$(NH_4)_2\,SO_4$ 0.1%

$Fe\,SO_4$ 0.001%

On Sept. 9th small plants of Phaseolus, grown in sand, and of equal size, were planted into these pots, two in each. After three weeks a considerable difference was noticed. In the three series the development was best, where the ratio $\frac{Ca\,O}{Mg\,O}$ was = 2.

The following table gives the measurements taken at this time :—

	$\frac{Ca\,O}{Mg\,O}$	A.	B.	C.
Length of stem, cm.	3	13.5	11.5	13.5
	2	18.5	17.0	15.5
	1	14.0	14.0	11.5
	0.50	13.0	15.0	10.0
	0.33	13.0	—	—
Length of the largest leaf, cm.	3	8.0	6.0	5.0
	2	9.0	7.3	5.5
	1	7.5	6.0	5.5
	0.50	6.0	6.5	5.0
	0.33	6.5	—	—
Breadth of the largest leaf, cm.	3	3.9	4.0	3.0
	2	4.9	3.8	3.2
	1	4.6	3.0	3.0
	0.50	2.8	3.5	2.5
	0.33	3.5	—	—

This table shows clearly not only an *influence of the ratio of* $\frac{Ca\,O}{Mg\,O}$ *upon the height of the plant but also on the size of the leaves. The best ratio is here 2 : 1*, at least before the fruiting stage of this plant. The plants No. V of B and C had died at the time the measurements were made, very probably from the excess of magnesium carbonate.

Unfortunately the experiment had to be terminated soon afterwards on account of fungi making their appearence on the leaves.

On the Behavior of the Phosphoric Acid in the Soils Towards Different Organic Acids.

BY

G. Daikuhara.

Many investigations have been carried out to determine how much phosphoric acid in a soil is available for the plant roots. Nearest to the truth came *B. Dyer* who published an elaborate investigation on "The Analytical Determination of probably available Mineral Plant Food in Soils," and proposed to apply a solution of 1% citric acid to determine whether a soil is in need of phosphatic manure. He suggested that "when a soil is found to contain as little as about 0.01 percent of phosphoric acid soluble in a 1 percent solution of citric acid, it would be justifiable to assume that it stand in immediate need of phosphatic manure."

I believed to be of some interest to compare citric acid with other acids in this regard and also to compare different soils.

I. *Application of organic acids in 1 per cent solution.*

The samples of soil were taken from the experimental paddy field (sandy loam) of the Kinai Branch of the Imperial Agricultural Experiment Station at Kashiwara, Osaka, which were manured every crop with different quantities of phosphoric acid during three years as follows:—

Upland Soil.	No. I.	No. P_2O_5
	No. II.	37.5 Kg P_2O_5 as Superphosphate per ha.
	No. III.	93.75 Kg P_2O_5 " " " "
Paddy Soil.	No. I.	No. P_2O_5.
	No. II.	37.5 Kg P_2O_5 as Superphosphate per ha.
	No. III.	112.5 Kg P_2O_5 " " " "

[1] Journ. of the Chem. Soc., London, *65*, 115 (1894).

Each plot had received moreover 112.5 Kilo N as NH_4Cl and 93.75 Kilo K_2O as K_2CO_3 per ha.

The extractions were carried out according to B. Dyer's method. The following table shows the result of analysis in the percentage of dry fine earth:—

			P_2O_5 Soluble in			
			1% acetic a.	1% tartaric a.	1% citric a.	1% oxalic a.
A.	Upland Soil.	No. I. No. P_2O_5	0.00832	0.04190	0.08381	0.12955
		No. II. 375 Kg P_2O_5	——	0.04798	0.09596	0.16929
		No. III. 93.45 Kg P_2O_5	0.01184	0.06078	0.09916	0.18001
B.	Paddy Soil.	No. I. No. P_2O_5	0.00096	0.00928	0.01823	0.04606
		No. II. 37.5 Kg P_2O_5	0.00128	0.01408	0.02495	0.05342
		No. III. 112.5 Kg P_2O_5	0.00192	0.01823	0.04526	0.09660

The weakest acid was therefore acetic, the strongest oxalic acid. In other cases, however, tartaric and citric acids extracted a little more than oxalic, as seen from the following table:—

SOILS OF Exper. Stations	Geological formation.	Character.	P_2O_5 Soluble in hot H Cl.	P_2O_5 Soluble in 1%			
				Acetic acid.	Tartaric acid.	Citric acid.	Oxalic acid.
Tokyo Central Station.	Diluvium.	Clayey.	0.37%	trace.	0.0068%	0.0115%	0.0061%
Kinai Branch[1] (Upland soil.)	Alluvium.	Sandy.	0.20%	0.0355%	0.0495%	0.0948%	0.1768%
Kinai Branch[2] (Paddy soil.)	"	Sandy loam.	0.08%	very little.	0.0170%	0.0248%	0.0562%
Tōō Branch.	"	Clayey.	0.10%	trace.	0.0133%	0.0245%	0.0414%
Sanyo Branch.	"	Sandy loam.	0.18%	0.0066%	0.0332%	0.0880%	0.1075%
Shikoku Branch.	"	Loam.	0.16%	0.0058%	0.0249%	0.0475%	0.1442%
Kiushu Branch.	Diluvium.	Clayey.	0.33%	trace.	very little.	0.0075%	0.0054%

[1] Upland field soil containing 31.54% fine earth.
[2] Paddy field soil containing 71.98% fine earth.

II. *Extraction of Soils with Organic Acids of Different Strength.*

The soil serving for these experiments contained 1.635% of hygroscopic water and 0.1727% of P_2O_5 soluble in boiling hydrochloric acid (sp. gr. 1.25). The method of extraction was also here that of Dyer.

The following table shows the results:—

Of the acids.	Acetic acid.	Tartaric acid.	Citric acid.	Oxalic acid.
0.25%	——	——	——	0.1326%
0.50%	——	0.0403%	0.0855%	0.1609%
1.00%	0.0355%	0.0495%	0.0948%	0.1798%
2.00%	0.0499%	0.0704%	0.1029%	0.1954%
5.00%	0.0586%	0.0918%	0.1173%	0.1964%

In this case the extractive power of oxalic acid for P_2O_5 in the soil was strongest, next in order came citric and tartaric acids, and finally acetic acid.

Can Boric Acid in High Dilution Exert a Stimulant Action on Plants?

BY

M. Nakamura.

It has been shown by various authors that the soil of certain districts contains small quantities of borates, hence also the plants grown on such soils contained some boric acid. *E. Hotter*[1] proved the presence of boric acid in many plants by extracting their ashes with water and transforming the boric acid into its methylic ether which was distilled off. It is especially the fruits in which the boric acid accumulates; in 10000 parts of the dry matter of fruits the amount of boric acid was found to vary from 2,2 to 12,8 parts.

Callison[2] made similar observations. Of some interest is also the observation of *Crampton*[3] that boric acid occurs in grapes grown in California. *A. Herzfeld* and E. v. Lippmann have made further observations on the occurrence of not insignificant traces of boric acid in lemons and other fruits. *F. Schaffer*[4] observed recently its normal occurrence in wines. *Hotter* determined to which extent boric acid and borates will exert a poisonous action on plants. When borates are added to the amount of one per mille to culture solutions, the growth was very much injured and the plants died after 20 days. Even an amount of 10 milligram boric acid per liter can exert some noxious action; some difference in resistance power was, however, noticeable with various plants.[5] The source of boric acid in the soils is probably turmalin which mineral contains about 10% of boric acid

[1] Jahresbericht f. Agricultur Chemie, 1890, p. 203, also Zeitschrift für Nahrungsmittel, etc, 1895.

[2] Jahresbericht f. Agricultur Chemie 1890.

[3] Jahresbericht f. Agricultur Chemie 1889.

[4] Schweiz. Wochenschr. Chem. Pharm. [1902.] *40*, p. 478.

[5] In regard to algae (Spirogyra, Vaucheria) Loew mentions in Flora, 1892, p. 374 that they are not injured within several weeks by adding 0.2 per mille boric acid to the culture water.

and frequently occurs in crystalline rocks and granular limestone. Since poisons exert a less powerful action in the absorbed condition in the soil than in the dissolved state in a culture solution, and moreover since poisons in small doses can exert a stimulant action, I observed cultures of barley in soil to which I added 10 milligr. and 50 milligrams respectively of borax per Kilo. These pots were manured with 1g. $NaNO_3$, 1gr. K_2CO_3 and 1.2 g. double superphosphate. Ten young barley shoots were planted, October 24, into each pot and after the young shoots had reached about 15 cm they were reduced to 4 of nearly equal height. The pots were kept in the glass house in which even in the late autumn the temperature on bright days reached sometimes 25°C. Measurements of the shoots to the tip of the longest leaves were made on Nov. 9, Dec. 1 and Dec. 12 with the following results:

Measurements Cm.

		Nov. 9	Dec. 1	Dec. 12
50 mg Borax	1	31	39,8	45
	2	27	35,5	42
	3	26	34	41
	4	28	38,5	46
	Average	28	36,8	43,5
Control	1	30	41	51
	2	24	37	45
	3	33	43	51
	4	28	37	40
	Average	29	39,5	46,75

The percentage of increase was therefore from Nov. 9 to Dec. 12 the following.

50 mg Borax: 1) 31 %; 2) 35,7%; 3) 36,5%; 4) 39,0% — average = 35,5%

Control: 1) 41,2%; 2) 46,6%; 3) 35,3%; 4) 30,0% — average = 38,5%

A photograph was taken on February 15. It is reproduced on Plate XXVIII and shows that 50 milligrams of borax acted very injuriously on the developement of barley, and even as little as 10 milligrams per kilo soil did some dammage. On February 16 were added 0,5 g. ammoniumsulphate in high dilution to each pot. On March 3 the control plants showed developement of three ears, while even 8 days later there was no sign of ears observed with the borax plants.

On April 23 the plants were harvested with the following results, showing an injurious action of even the small amount of 10 milligrams borax per kilo soil.

	Total wt.	Number of grains	Number of branches	Average length of branches
Control	45.0 g	132	8	63,5 cm
10 mg. Borax	28.2 „	30	4	58,0 „
50 mg. Borax	17,3 „	24	4	46,0 „

In the following experiments, commenced February 1, with pea and spinach the amount of borax was reduced to 5mg. and 1mg. per kilo soil respectively. 10 seeds were planted into each pot and the young shoots were reduced to 4 per pot in the case of the pea. On April 24 the following results were observed:

Pea

	Average length	Number of flowers
5mg. Borax per kilo soil	62 cm	2
1mg. Borax per kilo soil	86,25 „	6
Control	69,5 „	3

Spinach		
	Average wt. of plants	Average length of leaves
5mg. Borax per kilo soil.....	10,35 g.	38,2 cm
Control	7,2 g.	34,0 „

It will be observed that one milligram of borax per kilo soil exerted some stimulant action with the pea plants and 5mg. also with spinach plants.

The high degree of poisonous qualities of borax, which even injures plants in doses of 10 milligrams per kilo soil are certainly unexpected. This is of especial interest at present as, a discussion is now carried on as to the admissibility of borax for purposes of preservation of articles of food. In this discussion the remarkable poisonous character of borax for animals is pointed out. *F. Hofmann*[1] inferred from his experiments with dogs and rabbits that boric acid is "ein starkes Zellgift." *Rost*[2] observed that the body weight decreases continuously by the use of borax and vomition and diarrhea may result. *E. Kister*[3] and also *G. Merkel*[4] observed that 1—2 grams of boric acid can produce injuries of the stomach and diarrhea. Such an opinion was also expressed by *H. Mayer*.[5] On the other hand *Liebreich* and *Gerlach* deny the injurious charactar of borax and boric acid in small doses. However, when we take the highly poisonous character of borax for plants into consideration, we must admit also the dangerous character of borax for animals and man.

[1] D. Med. Wochenschr. 1902, No. 46.

[2] Ibid. 1903, February.

[3] Zeitschr. Hyg. 1901.

[4] M. Med. Wochenschr. 1903, No. 50, p. 100.

[5] Hyg. Rundschau 12, 1230.

It must also be mentioned here that *Doane* and *Price* reported that calves fed with milk containing borax lost their hairs. Marryland Agric. Exper. Station Report No. 86.

On the Action of Vanadin Compounds on Plants.

BY

S. Suzuki.

Although vanadin compounds occur very rarely in nature, vanadin was nevertheless discovered in the ash of the sugar beet by *Ed. O. v. Lippmann.*[1] Observations on the action of vanadin compounds on plants have not been made to my knowledge. It was, however, very probable that in moderate concentration they would act poisonously. Since poisons, however, often exert a stimulant action when applied in very high dilution, I have instituted a series of experiments.

In order to observe at first the degree of poisonous action, shoots of barley (20 c.m. high) were placed in solutions of vanadin sulphate[2] of 1%; 0.1% and 0.01 per cent. After 5 days the shoots in the solution of 1% were dead, while in that of 0.1% the leaves wilted. But the shoots in the 0.01% solution were still heabthy even after 12 days.

Water cultures in Knop's solution[3] were also started to which 1.0 and 0.1 per mille of that sulphate was added. A third experiment was made with a soil culture, 10 mg. of the hypovanadic sulphate being added per kilo soil in one pot, 50 mg. per kilo in a second. A third pot served as control. Each pot contained 10 kilo soil[4] and was sown on Dec. 13 with winter barley, 15 seeds in each, which were reduced to 7 of equal size in each pot on Jan. 20.

[1] Bert. Ber. Vol. 21, p. 3492.

[2] I applied the bluish green sulphate of commerce, the so called hypovanadic sulphate, $V_2O_2(SO_4)_2$. This salt has a strong acid reaction.

[3] Only the amount of magnesium sulphate was a little increased.

[4] Each pot was manured with ammonium sulphate 2.3 g., sodium nitrate 2 g., potassium sulphate 2 g., sodium phosphate 2.5 g. and sodium chlorid 1 g.

Water culture. Barley shoots (16–17 cm. high) were placed (Dec. 4) in 6 flasks.[1]

a and a_1 received 0.1 per mille vanadin sulphate.

b and b_1 „ 0.01 „ „ „ „

c and c_1 served as control.

The observations made on Jan. 20 were as follows:—

	Lengh of the longest leaves.	Number of stalks: thick	Number of stalks: thin	Number of leaves: living	Number of leaves: dead	Length of the roots.	Remarks.
a	16.0 c.m.	1	5	4	9	2.0 c.m.	No root hairs visible. Development stopped; fresh leaves appear but the old leaves die off.
a_1	15.5 „	1	3	5	8	1.5 „	
average	15.8 „	1	4	5	9	1.8 „	
b	21.0 c.m.	5	3	22	2	20.0 c.m.	Normal.
b_1	21.0 „	5	1	17	3	20.0 „	
average	21.0 „	5	2	20	3	20.0 „	
c	18.0 c.m.	6	2	21	3	17.5 c.m.	Normal.
c_1	23.0 „	4	2	15	3	10.0 „	
average	20.5 „	5	2	18	3	13.8 „	

A very weak stimulant action on the roots seemed to have taken place in the case b and b_1. But in the cases a and a_1 the poisonous action was so decisive that the plants were no longer observed. The final observations were made on Feb. 27 with the following results:—

	Length of the longest leaves.	Number of thick stalks.	Number of thin stalks.	Number of living leaves.	Number of dead leaves.	Length of the roots.	Weight in a fresh state: roots	Weight in a fresh state: upper portion
b	32.5 c.m.	21	1	76	10	25	39.7 g.	27.4 g.
b_1	30.0 „	18	2	70	8	25	37.7 „	28.3 „
average	31.3 „	20	1.5	73	9	25	38.7 „	27.9 „
c	34.0 c.m.	21	1	76	13	30	37.7 g.	25.9 g.
c_1	33.0 „	18	1	66	10	23.5	42.7 „	28.2 „
average	33.5 „	20	1	71	12	27	40.2 „	27.1 „

This experiment proves that in a normal water culture barley is very much injured by the addition of 0.1 per mille vanadin sulphate, and further

[1] The solutions were renewed on Dec. 20, Jan. 8, 20, 31 and Feb. 21.

that when applied in the further dilution of 0.01 per mille no decisive stimulation takes place, although no injurious action is any more exerted.

Soil culture. The shoots above mentioned were measured several times. The observations were as follows:—

	Average length of the longest leaves. Jan. 23.	March 27.	April 10.	Average number of stalks. March 27.	April 10.
	c m.	c.m.	c.m.		
Pot I. (0.1 g. vanadin sulphate per 10 kilo soil.)	8.6	26.4	57.7	3	3
Pot II. (1 g. „ „ „)	8.9	30.3	59.1	3	3
Pot III. (Control)	9.1	37.2	62.8	4	4
Pot IV. („)	9.3	33.1	63.5	4	4

This experiment plainly shows that vanadin sulphate even in a very small quantities has no stimulating action on barley.

Can Potassium Ferrocyanid Exert any Stimulant Action in the Soil on Plant Growth?

BY

S. Suzuki.

In a former article I have shown that potassium ferrocyanid even in a very high dilution acts poisonously on plants in water culture.[1] The question, however, seemed to be of some interest whether this compound could exert a stimulant action when incorporated in a small quantity into the soil.

Four Wagner's pots each containing 10 kilo soil served for the experiment. Each pot received as manure:—

Ammonium sulphate	2.3 g.
Sodium nitrate	2.0 „
Potassium sulphate	1.9 „
Sodium phosphate (cryst.)	2.5 „

Two pots served as control while one pot received 0.1 g. potassium-ferrocyanid and another 1 g. Fifteen seeds of barley were sown in each pot on Dec. 13. and the young shoots reduced to 7 of equal size on Jan. 20. After a few weeks a decided difference was noticed in favor of the pot that received 1 g. potassium ferrocyanid. Measurements were made on March 7 and 27 with the following results:—

[1] These Bul. Vol. V, No. 2.

	Average length of the longest leaves.		Average number of stalks.	
	March 7.	March 27.	March 7.	March 27.
Pot I. (0.1 g. K_4 Fe Cy_6).	14.4 c.m.	31.2 c.m.	5	5
Pot II. (1 g. K_4 Fe Cy_6).	26.8 „	44.6 „	4	5
Pot III. (Control).	12.8 „	37.2 „	4	4
Pot IV. („).	15.6 „	33.1 „	4	4

The question arised whether the favorable effect in pot II was due to the potassium ferrocyanid as such or to the nutritive action of its decomposition products. It was possible that the soil bacteria decomposed the salt, whereby the iron was liberated as ferric hydrate,[1] nitrogen as ammonia and potassium as carbonate. In order to decide this question 20 g. of the soil of the pot II were extracted on March 17 with water and the filtrate tested with ferric chlorid, but only an exceedingly feeble reaction was obtained. In a second test diluted hydrochloric acid served for the extraction, but with no better result. A control test with 100 g. unmanured soil moistened with a dilute solution of 10 m.g. potassium ferrocyanid showed further that this small quantity is entirely absorbed. It remains therefore for the present undecided whether in the case above mentioned the potassium ferrocyanid acted favorably as a stimulant or by the products of its decomposition as a source of nutrients.

[1] Previous experiments had shown that a small addition of ferrous sulphate to the soil in question increases the yield of rice and of oats.

Are Soluble Iodids Absorbed by the Soil?

BY

S. Suzuki.

My experiments on the stimulating action of potassium iodid on agricultural crops[1] made it desirable to know whether the soil can retain iodids in a certain measure by absorption. In regard to chlorids, absorption by adhesion has been observed by various authors. The interesting experiments by *B. Dyer*[2] on the field of *Rothamsted*, e. g., have shown that chlorids are to a certain degree retained by clay soils. He writes: "Now the average quantity of chlorine which falls annually in the rainfall at Rothamsted, as calculated on observations for 22 harvest years, 1877–1878 to 1898–99, was 14.75 pounds." "Yet we see that the soil of plat 5 in the Broadbalk wheat-field retains, on the average, within each depth of 9 inches down as far as 90 inches, a quantity of chlorine equivalent to that which falls upon its surface each year in the form of rain.[3] In other words, down to a depth of 90 inches the soil, though continually subjected to the washing influence of the rain, contains a quantity of chlorids equivalent to that falls upon it during ten years, neglecting the very few pounds annually supplied to it as impurities in the mauures." "It would seem that the clay enters into some sort of combination with the chlorids from which they are only dislodged by a very free application of water." "The difficulty of

[1] These Bulletins Vol. V. No. 2 and p. 474 in this number.

[2] Office of Experiment Stations, Bul. No. 106, U. S. Depart. of Agric. p. 82 and 83.

[3] These quantities are in certain countries comparatively large. Thus in *Barbados* were found by *Albuquerque* per million parts of rain water from 6 to 38.5 parts of chlorine, while the nitrogen as ammonia varied between 0.015 to 0.212. (Report of the Agric. work in Barbados, Government Exp. Station, 1902.)

removing chlorids from the soil by percolation except when a relatively very large quantity of water was used, was demonstrated in some experiments described in the paper on the rain and drainage waters at Rothamsted."

In my experiments with potassium iodid I compared the behavior of this salt in the soil with that of potassium chlorid, 1 per mille solutions of both these salts serving for the filtration through the soil. As reagent for iodine served starch paste to which freshly neutralized hydrogen peroxid and a trace of ferrous sulphate was added. By this reaction of *Schönbein* very small traces of iodine can be discovered, in the form of the blue iodine starch. First test:—

The stratum of soil was 8.5 c.m. high and 5.8 c.m. wide. 200 cc. of each solution were poured gradually on the surface of the soil contained in a cylindrical vessel. After 35 minutes the first drops appeared at the lower end. While now in the case of potassium chlorid already the first 2 cc. showed a moderate and the second 2 cc. a considerable reaction for chlorine[1] with silver nitrate, there was no iodine reaction obtained in the first 25 cc. of the filtrate. After this a moderate reaction appeared in the next 6 cc. and a strong reaction in the following 2 cc.

Second test:—Here the column of the soil was higher, namely 15 c.m., but the diameter was smaller than in the former case, namely 3 c.m. While the weight of the fine soil used in the first case was 200 g., it was here only 84 g. The solutions were added in this case in such a manner that the surface of the soil was constantly covered by it in a height of 2 c.m. The total quantity of solution added was 100 cc. After about one hour the first drop appeared at the lower end, and while the chlorine reaction was obtained with the first 2 cc. there was no iodine reaction noticed in the first 15 cc. After this the next 3 cc. showed a weak and the following 3 cc. a strong reaction for iodine. Both tests proved decisively that an iodid is much better absorbed in the soil than a chlorid. The calculation shows for

[1] A control test was made with a distilled water free from chlorine. The first few cc. of this filtrate showed a weak reaction for chlorine owing to the chlorid already present in the soil, but the turbidity was much lighter than in the case of potassium chlorid solution.

the first experiment that 100 g. soil absorbed 0.0125 g. potassium iodid and in the second case 0.018 g. The effect depends naturally much on the height of the soil stratum.

Fig I

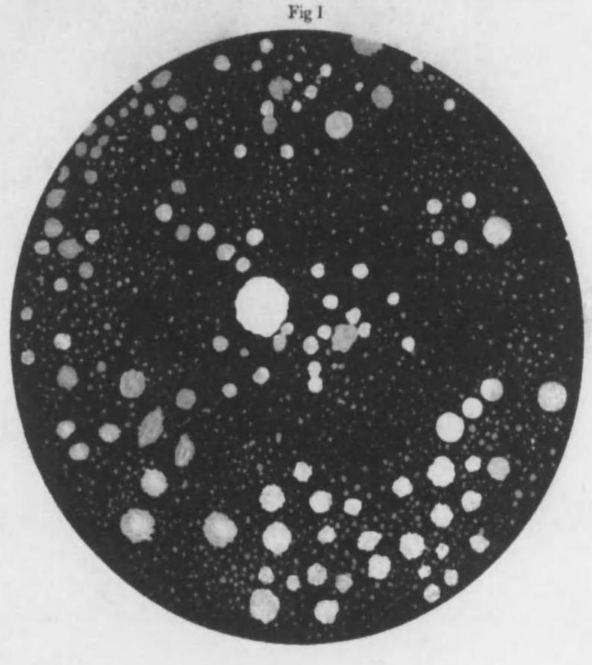


Fig II

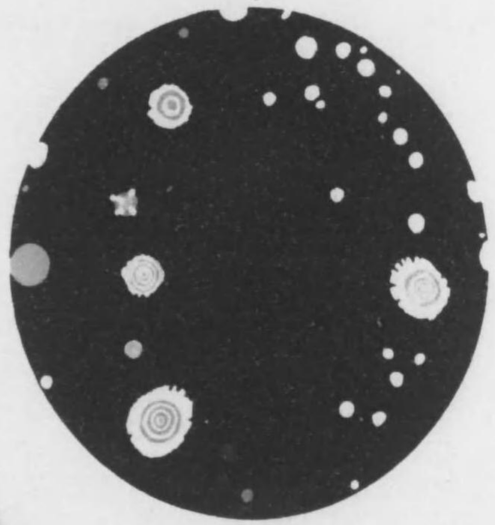
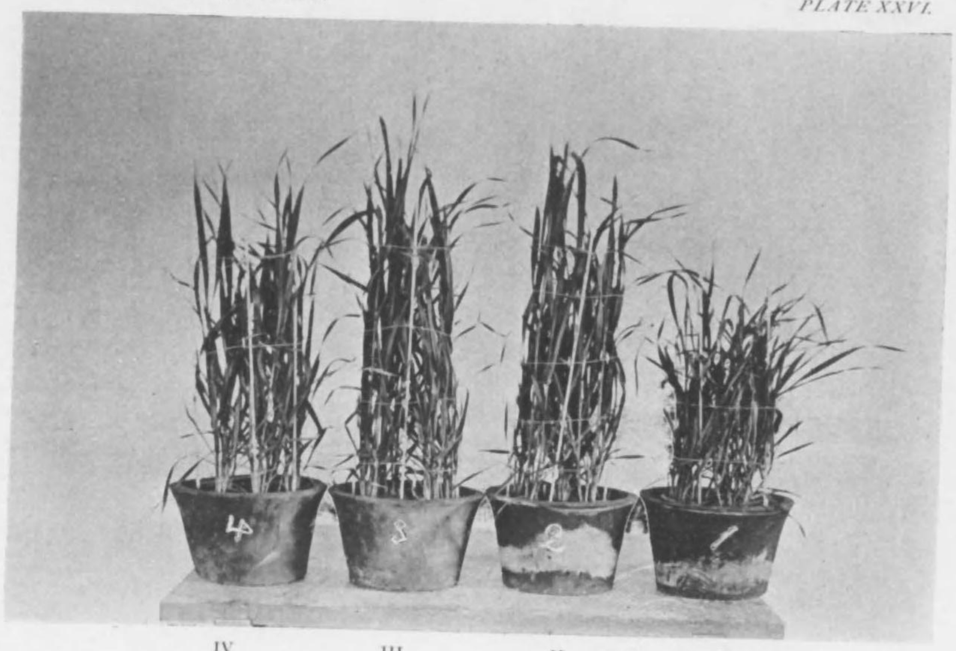


Fig. I. Agar-plate from the digestive juice of a silk worm, 30 days at room temperature. Original plate.

Fig. II. The same. Second dilution.

I II

Plate showing the stimulating action of rubidium chlorid upon barley.
I Rubidium plants. II Control plants. To page 464.



IV III II I

Plate showing the influence of iodid of potassium on oats. I, II and III, Iodine plants; IV, Control plants.

To page 474.

Plate showing the influence of different ratios of lime and magnesia upon mulberry plants. To page 499.

I II III

Plate showing the injurious action of borax on barley. I, 0.05 g borax per Kilo soil. II, 0.01 g borax. III, Control. To page 511.

東京帝國大學

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