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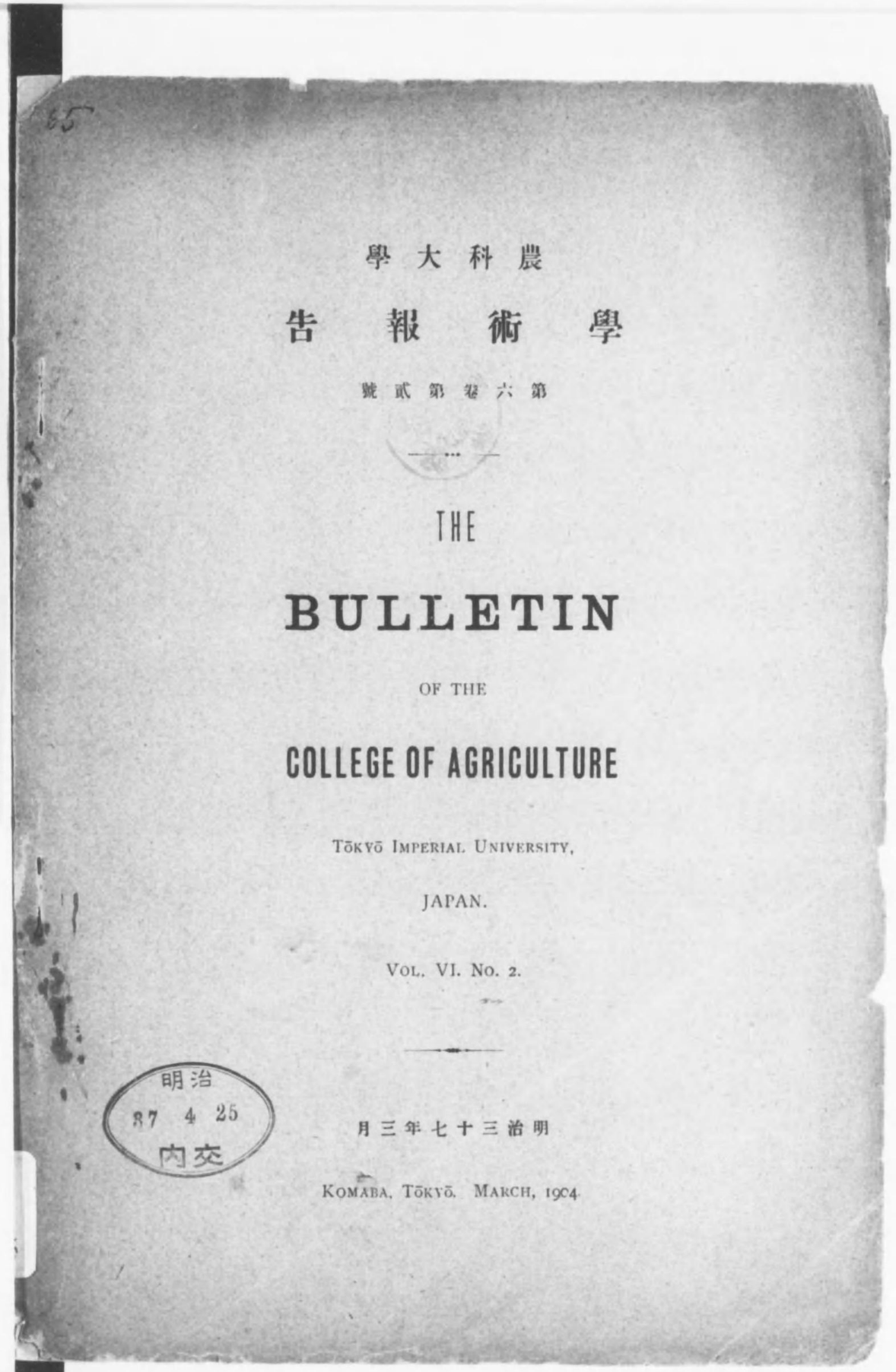

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Studies on the Lability of Enzyms.

BY

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The cause of the chemical powers of enzyms has frequently been the object of speculation. According to the theory of O. Loew,[1] this activity is intimately connected with the lability of the enzyms, in as much there exist in them certain labil groupings which exert chemical energy—a kind of atomic motion—which can cause chemical changes in certain other compounds. A condition for the action of enzyms is that the compound to be acted upon, shows a certain configuration as was shown by *E. Fischer*.

Various compounds can destroy the activity of enzyms what can be explained by their causing the migration of atoms from the labil to the stable position within the enzym molecule. But, in most of such cases no conclusion can be drawn as to the nature of the labil groups. Thus, for instance, carbonate of soda in 1 per mille solution will soon destroy the action of pepsin and takadiastase. This is a special case of the phenomenon that alkalies and acids can change various labil compounds to stable ones. In order to be able however to draw certain conclusions as to the *chemical nature of the labil groupings* we must select such compounds that have quite specific actions, even in high dilution and in perfect neutral solution. *Loew* suspected formerly that the lability of enzyms is caused by the simultaneous presence of amido—and aldehyde groups, but his own tests with alkaline silver solution failed.[2] The presence of aldehydegroups would provide a plausible view, as *Vernon*[3]

[1] Pflüg. Arch. 27., 212; Die chemische Energie der lebenden Zellen, p. 149; Journ. f. prakt. Chem. 1888, p. 194.

[2] Pflügers Archiv. für die ges. Physiologie, vol. 27, p. 212.

[3] Journ. of Physiology, vol. 29, p. 331 [1903].

has pointed out: "it may, for instance by alternate hydration into $CH(OH)_2$ groups and subsequent dehyration be able to effect the hydrolysis of proteids, whilst di-amido-or other aldehyde groups by the reverse process may be able to effect the dehydration of caseinogen into casein." As to the action of zymogens, *Vernon* expresses himself as follows: "Let us also provisionally accept *Loew's* hypothesis that ferments differ from inactive proteids in virtue of their containing aldehyde groups. Then we may assume that the formation of ferments in the cells of digestive glands consists in the activation of ordinary proteid molecules by the reduction of some or all of their COOH or acid groupings into CHO or aldehyde groupings."

It is also possible that according to a later view of *O. Loew*, the zymogens contain ketonegroups, and that the activation process consists in the opening of lactamgroups in the zymogen molecule, labil amido-groups thereby being generated.[1] Amidoketones also are very labil bodies as seen from the behavior of diamidoacetone which spontaneously changes to an indifferent substance (*Rügheimer* and *Mieschel*). In regard to the amidogroups O. Loew infers their presence from his observation that dilute formaldehyde easily destroys the action of enzyms at the ordinary temperature.[2] It is well known that formaldehyde easily attacks amido-groups of a certain lability, e.g.,:

$$C_6H_5.NH_2+CH_2O=C_6H_5.N=CH_2+H_2O$$

Thus if labil amidogroups in enzyms would be changed in an analogous manner, the activity of this grouping would of course be lost, since the amidogroup as such has disappeared. The following table shows the more or less intense action of formaldehyde on enzyms.

[1] Centralbl. f. Bakteriologie 12, p. 445.

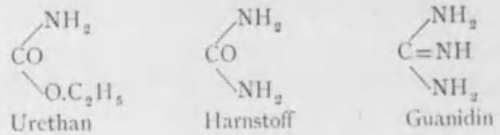
[2] Journal für prakt. Chemie 1888, vol. 37. p. 104. Such observations were later on made also by various authors.

Enzym.	Formaldehyde.[1]	Time in which the enzym is killed.	Author.
Diastase	1%	in 24 hours	Bokorny.
Myrosin	5%	soon	"
Rennet	0.5%	"	"
Zymase	0.05%	"	Wroblewsky
Sucrase	5%	one hour at 54° C.	Pottevin
Catalase	4%	1 hour	Loew
Pepsin	5%	24 hours	"
Pepsin	4%	24 hours	Sawamura
Papain	0.4%	Soon at 40°	Vines

It is true that formaldehyde will act also on hydroxylgroups and produce methylene-compounds as, e.g., with the polyvalent alcohols, yielding the so-called "formals," but in order to accomplish this, application of heat in presence of hydrochloric acid is required, hence the conditions are far different from those just mentioned. The inference that amidogroups of a certain lability are concerned in the activity of enzyms would receive further support, if it could be shown, that the enzyms take

[1] The commercial formalin or formal contains about 40% formaldehyde.

up free cyanogen and would lose thereby their activity.[1] As to the different lability of amidogroups *Loew* expresses himself as follows: "Die Amidogruppe kann unter gewissen Umständen stabil, reactionsunfähig, unter anderen aber wieder äusserst labil und reaktionsfähig sein. Die Amidogruppe ist Z. B. im Urethan sehr stabil, im Harnstoff labiler, noch mehr im Guanidin. Im Hydroxylamin und Diamid aber ist sie so energisch geworden, dass diese Stoffe selbst bei grosser Verdünnung noch in alles lebende Protoplasma ohne Ausnahme eingreifen können, d. h. Gifte allgemeinen Charakters sind. Folgende Formeln lassen die Einflüsse benachbarter Gruppen auf die Energie der Amidogruppe erkennen:"



Urethan

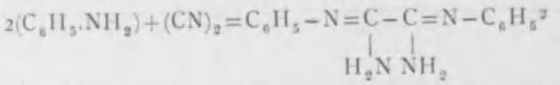
Harnstoff

Guanidin

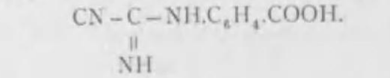
Diamid (Hydrazin)

Hydroxylamin

When dicyanogen acts on amines, it forms as chief compounds addition products with two molecules of amine. Thus



When, however, it acts on amidocompounds several products may result. Thus the chief product with amidobenzoic acid



[1] Also hydroxylgroups can take up cyanogen at the ordinary temperature as *Loew* has shown with pyrogallol. But this is the only case known thus far. (Journ. f. prakt. Chem. Vol. XV. 326.) Very labil methylene groupings as in the acetylacetic ether also can react with cyanogen but only in presence of sodium ethylate C_2H_5ONa (W. Traube). Thus far, however, only a few such cases have been described.

[2] This formula has been shown by *Tiemann* to correspond better to the behavior of the cyananiline than the former imidoformula.

It is noticeable, however, that not every amido compound and amide can combine with dicyanogen, and imidogroupings are not acted upon at all. Neither hydrazobenzol nor asparagine nor urea are acted upon. But *Andreasch* has shown that methylthiourea enters into reaction. The peculiar behavior of free cyanogen towards highly labil amidogroupings have induced *Loew* and *Tsukamoto*[1] to test the behavior of a highly diluted aqueous solution of dicyanogen towards living organisms of the most different kind. These tests have revealed highly poisonous properties of dicyanogen rendering the presence of labile amido groupings in the proteins of living matter highly probable. It was now of interest to test whether it could also kill the enzyms. Since most enzyms belong in all probability to the protein group it could hardly be doubted that dicyanoger. would act on enzyms when applied in excess to a concentrated solution, since *Loew* has shown that cyanogen combines with ordinary albumin.[2] He applied solutions containing 10–25% albumin. In my experiments with enzyms, the dilution was a much higher one, since it was to be expected that very labile amido groupings would take up dicyanogen in high dilution, hence a reaction under this condition would admit a safer conclusion as to the degree of lability.

Experiment with Pepsin.

2 grams of commercial pepsin[3] were dissolved in 200 c.c. water and divided into halves.[4] Cyanogengas developed from 5 grams mercuric cyanid was passed through one of these bottles which, well closed, was left for 12 hours. Thereupon 10 c.c. of 2% hydrochloric acid were added and some fibrin, previously swollen in dilute hydrochloric acid, and kept for 24 hours at 28° C. The fibrin was dissolved rapidly in both cases

[1] Forschungsberichte über Lebensmittel, Vol. I. No. 7;—Journ. College of Science, Tokyō, 1896. Cf. also These Bulletins Vol. II. No. 7.

[2] Journ. f. prakt. Chem. 1877.

[3] The solution of this sample was acid.

[4] Some ether was added to the control flask to prevent bacterial growth.

and neither the precipitation with nitric acid nor the saturation with ammonium sulfate did show any difference; nor the colorimetric comparison of the biuret reaction. In a second experiment, 2g of pepsin were dissolved in 200 c.c. of water and 20 c.c. of a 2% hydrochloric acid added. One half served as control, while the other half was treated with the same quantity of cyanogengas as before, but in this case, the solution was kept at 35-40°C during the treatment. Moreover this liquid was placed in the incubator at 28°C for 20 hours before testing its proteolytic action. Equal quantities of fibrin and thin square slices of boiled egg white were now added to both liquids which were kept at 28°C for one hour. The fibrin was dissolved also here and the egg-albumin was almost wholly digested after 3 hours in both cases. In the third experiment the conditions were again changed. Pepsin, 1g, was dissolved in 200 c.c. water and 1g of sodium carbonate (anhydrous) added. 100 c.c. of this solution were treated with the same quantity of cyanogen as before and left for 12 hours. After neutralizing 10 c.c. of 2% hydrochloric acid were added and fibrin. The result showed that the pepsin had been killed by the sodium carbonate. This result is not surprising considering the great sensitivness of pepsin toward alkaline liquids. *Green* reports that pepsin is injured by 0.002% soda solution after 1-2 hours at bodily temperature.[1] Neverthless, a further experiment was made with the modification that the pepsin solution was rendered but very slightly alkaline with sodium carbonate. The passing of cyanogen from 5 grms of mercuric cyanide on heating took about one hour. Afterwards 10 c.c. of 2% hydrochloric acid were added to 100 c.c. of pepsin solution, the treated one, as well as the control. The same quantity of fibrin was added in both cases, but no digestion took place in either case. In the final experiment the acidity was hardly perceptible to litmuspaper; the further treatment was the same as just mentioned. In both cases, the fibrin was quite dissolved after one hour while the slices of boiled egg disappeared after 12 hours.

[1] *Langley* and *Eves* found a distinctly inhibitory action to be manifested by the presence of as little as 0.0015% of sodium carbonate.

Experiment with Trypsin.

1 gram of commercial trypsin was dissolved in 200 c.c. of water containing 0.4g. sodium carbonate and divided into halves, one serving as control and the other being treated with cyanogen gas developed from 5 grms of mercuric cyanid. After 12 hours an equal quantity of fibrin and two thin square slices of boiled egg were put in each solution. After two hours at 28°C the fibrin was almost completely digested in both solutions, also the egg slices were very much attacked, but did not disappear completely after 20 hours. In the next experiment, the quantity of mercuric cyanid was doubled, but the result was the same as before.

Experiment with Emulsin.

A solution of 0.1% emulsin with 0.1% Na_2CO_3 was treated with cyanogen gas developed from 5 grms mercuric cyanid. After standing for 48 hours, 0.1g. of amygdalin was added to 10 c.c. of the solution. After one hour at 28°C, the peculiar odor of benzaldehyde was plainly perceptible like in the control case. The decomposition of amygdalin was also shown by the reaction with ammoniacal silver solution and with fuchsin solution decolorized by sulphurous acid, proving the formation of the decomposition products of amygdalin, viz. of glucose as well as of benzaldehyde.

Experiment with Takadiastase.

0.2g. of commercial takadiastase were dissolved in 200 c.c. of water. This solution had a faint acid reaction and was rendered faintly alkaline by sodium carbonate. One half was treated with cyanogen developed from 5 grms mercuric cyanid while the other half served as control. After 24 hours standing the amylolytic action was compared. 10 c.c. of these solutions were mixed with 10 c.c. of 0.1% starch paste suspension

and kept at 30°C for 2 hours. On addition of iodine solution, no blue reaction set in, showing that the starch was transformed equally well in both cases.[1] On boiling with Fehling's solution, a strong sugar reaction was obtained and upon warming with ammoniacal silver solution, a silver mirror appeared in both cases. The enzym had therefore not lost its activity by the treatment with cyanogen.

Experiment with Oxidases.

45g. of a fresh radish root were triturated with addition of 100 c.c. water. Through 50 c.c. of this extract, cyanogen gas developed from 5 grms mercuric cyanid was passed while 50 c.c. served as control. After standing 15 hours, the cyanogen gas was replaced by air and the liquid tested for oxidizing enzyms in the usual manner, but the treated solution gave the color tests much weaker than the control. The experiment was repeated with the result, that when after 24 hours standing the color tests were made, they failed almost completely. Since dicyanogen in aqueous solution soon forms some prussic acid it was possible that some prussic acid had paralyzed the action of the oxidizing enzyms. Hence in the next trial the treated liquid was left to evaporate at 40-50°C to remove the prussic acid, whereupon the guaiac and the guaiacol test for peroxidase were readily obtained, only the guaiac test for oxidase was somewhat weaker.

In all the cases here described *dicyanogen has failed to destroy the activity of the enzyms*, what reveals a great chemical difference between the lability of enzyms and the lability of the active proteins in the living protoplasm.

Loew and Tsukamoto (l. c.) have observed that a fresh solution of dicyanogen in water in a dilution of 1 : 5000 kills bacteria and of 1 : 10000

[1] Although this solution had a weak alkaline reaction, the diastase was not perceptively injured. *Chittenden* and also *Grützner* have observed an injurious action of small quantities of alkalies, but *Epstein* and *Schulze* found that hereby this enzym is not destroyed, but merely "paralysed," because by neutralisation the activity is again restored, at least partially.

phaenogams, algae and lower aquatic animals. Here exists then another striking difference in the chemical behavior of living protoplasm and enzyms.

The apparent indifference of enzyms to dicyanogen shows either that the amido groupings in the enzyms are not of sufficient lability or that they are protected by other neighboring atomic groupings, forming a steric obstacle in the molecule. The inference that there are no amido groups present at all would be improbable considering the behavior of enzyms towards formaldehyde. Further tests therefore seemed necessary to demonstrate the participation of the amidogroups in the activity of the enzyms. Here the behavior towards nitrous acid promised to furnish some clue. In my experiment with enzyms I added to highly diluted solution of sodium nitrate and sodium nitrite the theoretically necessary quantity of sulphuric acid.[1] With those enzyms which are injured very easily by any kind of acids special care was necessary to apply nitric and nitrous acid in a very high dilution. The cause of the injurious action of nitric acid would of course be very different from that of nitrous acid. The former in high dilution would act like dilute sulphuric acid causing atomic migration of labil atoms while nitrous acid would act on labil amido groups in the following manner:

$$(x)NH_2 + N\begin{matrix}=O\\ \diagdown OH\end{matrix} \rightleftharpoons (x)-N=N-OH + H_2O$$ and

in the case of aliphatic compounds development of nitrogen would immediately follow the formation of a diazocompound:

$$\underbrace{(x)-N=N-OH}_{\text{Diazocompound}} \rightleftharpoons (x)-OH + N_2$$

Experiment with Pepsin.

1). 0.3g of pepsin were dissolved in 300 c.c. water, and divided into three equal parts. To one potassium *nitrite* and sulphuric acid in

[1] In this connection it is also an interesting fact that a very high diluted nitrous acid (1 : 100000) is much more poisonous for lower organisms than nitric acid, as *Loew* and *Bokorny* have shown.

high dilution were added in the calculated proportions to produce free nitrous acid of 0.1% in the liquid. To another potassium *nitrate* and sulphuric acid were added in such proportion as to produce 0.1% nitric acid, while the third portion served as control. The nitrous acid soon caused a yellowing of the pepsin solution, while nitric acid did not. After standing 24 hours,[1] an equal quantity of fibrin previously swollen in dilute hydrochloric acid and washed, further two square slices of boiled egg white and 10 c.c. of a 2% hydrochloric acid were added.

	After $^1/_2$ hour at 40°C.	After 1 hour at 40°C.
Control.	Fibrin dissolved; egg white not yet.	Egg white wholly dissolved.
Nitrous acid.	Fibrin dissolved; egg white almost unchanged.	Some egg white still remained.
Nitrous acid.	Some pieces of fibrin dissolved; egg white remained intact.	Egg white not attacked.

An equal quantity of fibrin was again added;

	After $\frac{1}{2}$ hour.	After 1 hour.	After 17 hours.	After 3 days.
Control.	Fibrin and egg white dissolved.	——	——	——
Treated with nitric acid.	Fibrin as well as egg white attacked.	Both almost dissolved.	——	All dissolved.
Treated with nitrous acid.	Fibrin and egg white not dissolved.	Both unchanged.	Fibrin dissolved, but not egg white.	Egg white a little attacked.

2). In a second experiment 0.2% nitric acid and nitrous acid were applied in the same way as before. In the control solution however, 0.2% sulphuric acid was added to show the effect of the mere acidity. The solutions were kept at the ordinary temperature for 24 hours.

[1] The solution treated with nitrous acid developed a peculiar odor of certain nitro-compounds.

Hereupon 10 c.c. of a 1% hydrochloric acid were added to each solution further an equal quantity of fibrin and two square slices of boiled egg white. These solutions were kept at 40°C.

	After $\frac{1}{4}$ hour.	After $\frac{1}{2}$ hour.	After 1 hour.	After 2 days. (at ordinary temp.)
0.2% sulphuric acid.	Fibrin dissolved very little.	Fibrin almost dissolved; Egg white unchanged.	Fibrin dissolved. Egg white unchanged.	Egg white dissolved.
0.2% nitric acid.	Fibrin little dissolved.	Fibrin almost dissolved. Egg white unchanged.	Fibrin dissolved. Egg white unchanged.	Egg white dissolved.
0.2% nitrous acid.	Not dissolved.	Some fibrin dissolved. Egg white not.	——	Some fibrin and egg white still unchanged.

A further quantity of fibrin and egg white was added and kept at 40°C:

	After $\frac{1}{2}$ hour.	After 1 hour.	After 20 hours.
0.2% sulphuric acid.	Fibrin almost dissolved. Egg white unchanged.	All fibrin dissolved. Egg white hardly unchanged.	Egg white dissolved very much.
0.2% nitric acid.	Fibrin almost dissolved. Egg white unchanged.	All fibrin dissolved. Egg white hardly unchanged.	ditto.
0.2% nitrous acid.	Much fibrin unattacked. Egg white unchanged.	Much fibrin and all Egg white unchanged.	Egg white unchanged; Some fibrin still present.

3). In the third test, 0.1g. of nitrous, nitric and sulphuric acid were added respectively, each bottle holding 100 c.c. of 0.1% pepsin solution, and immediately heated at 35°C for one hour. 10 c.c. of 1% hydrochloric acid, further eggwhite and fibrin were then added.

	After 2 hours at 40°	After 24 hours at 40°
0.1% sulphuric acid.	Fibrin dissolved.	Egg white almost dissolved.
0.1% nitric acid.	"	"
0.1% nitrous acid.	Some fibrin unattacked.	Egg white unchanged.

0.1 grm. of nitrous, nitric and sulphuric acid was again added to these solutions respectively, and kept at 40° for one hour. An equal quantity of fibrin was then added.

	After 1 hour at 40°
0.2% sulphuric acid.	All fibrin and all egg white dissolved.
0.2% nitric acid.	" " "
0.2% nitrous acid.	All fibrin and egg white undissolved.

Experiment with Trypsin.

Into three flasks holding 100 c.c. of a 0.5% trypsin solution, 0.05 grams of nitrous, nitric and sulphuric acid were added and kept at 40° for one hour. These solutions were now neutralized and 10 c.c. of a 2% sodium carbonate solution added and some fibrin.

	After 2 hours at 40°	After 17 hours at 40°
0.05% sulphuric acid.	Some fibrin dissolved.	All fibrin dissolved.
0.05% nitric acid.	" " "	" " "
0.05% nitrous acid.	Fibrin undissolved.	Fibrin undissolved.

Experiment with Emulsin.

This test was made in the same way as in the last mentioned case. After 20 hours, 10 c.c. taken from each flask received 0.1g. of amygdalin. After 15 minutes at 40°C, the following was observed.

Control.	Odor of benzaldehyde and prussic acid. Reduction with Fehling's solution and with ammonical silver solution.
0.1% nitric acid.	No odor developed. No reduction took place with the above-named reagents.
0.1% nitrous acid.	" " "

The result did not differ when the amygdalin was added after neutralisation of the liquids.

2). In the next experiment, the quantity of nitric and nitrous acids was reduced to 0.05%, while to the control solution sulphuric acid was added in the same concentration. After keeping for 16 hours at 18° these solutions were neutralized and 0.5g. amygdalin added. On keeping these mixtures now at 40° for three hours, the following was observed:

0.05% sulphuric acid.	The characteristic odor of benzaldehyd appeared very plainly.
0.05% nitric acid.	" " "
0.05% nitrous acid.	No odor developed.

These tests with pepsin, trypsin and emulsin show therefore, that nitrous acid destroys the activity of these enzyms more easily than do nitric and sulphuric acids in the same high dilution.

In order to test for *ketone groups*, experiments were made with hydrazine, methylhydrazine and hydroxylamine. The solutions of the salts of these bases having an acid reaction were neutralised with sodium carbonate. From the amount of salt weighed out, the amount of the free bases was calculated.

1) *Hydrazine.*

Pepsin: 100 cc. 0.1% pepsin solution + 1% free hydrazine.

After two hours at 40° 0.2% hydrochloric acid and three flocculi of fibrin were added and kept again at 40°C.

	After ½ hour.	After 2 hours.	After 4 hours.
1% free hydrazine.	Fibrin not attacked at all.	Not dissolved	Not attacked at all.
Control.	Fibrin wholly dissolved.	——	——

Trypsin: 1% trypsin solution + 1% free hydrazine.

After two hours at 40°C, 0.2% sodium carbonate and three flocculi of fibrin were added, keeping the mixtures in the incubator.

	After 2 hours.	After several days.
1% free hydrazine.	Fibrin unattacked.	Not attacked.
Control.	Almost dissolved.	All dissolved.

Diastase: Solution of 0.1% diastase + 1% free hydrazine.

After keeping at 40°C for 2 hours some 0.1% starch paste was added and kept at 40°C, for 2 hours. After evaporating and removing the hydrazine with alcohol the residue was treated with water and tested with iodine dissolved in potassium iodid.

1% free hydrazine.	Blue starch reaction.
Control.	No starch reaction.

Emulsin: 0.1% emulsin solution + 1% free hydrazine.

After keeping at 40°C for 4 hours, 0.1 grm. amygdalin was added to 10 c.c.

1% free hydrazine.	The odor of benzaldehyd and prussic acid developed very weak.*
Control.	The odor was very strong.

These solutions were kept at 40°C and tested in the same way again:

	After 6 hours.	After 8 hours.
1% free hydrazine.	A very weak odor appeared.	Trace of odor.
Control.	Very strong odor.	Very strong odor.

I have observed further that also zymase is easily killed by a 1% solution of hydrazine.

2) *Methylhydrazine.*

I. *Experiment.*

Emulsin, Pepsin } 0.2% solution + 0.032% free methylhydrazine.

Trypsin 0.5% „ + „

After keeping at 40°C for 1 hour was added:

To emulsin: 0.1g. of amygdalin

„ Pepsin: 0.2% HCl

„ Trypsin: 0.2% Na_2CO_3

Emulsin: After keeping at 40°C for 15 min. the enzym was still active but weakened somewhat by methylhydrazine.

* It might be objected, that the diminution of the odor might have been due to the formation of benzyliden hydrazine but the control tests with the fresh mixture and that which had been tested after 8 hours digestion at 40° revealed a great difference in the intensity of the odor. The formation of benzyliden hydrazine from benzaldehyde and hydrazine in such high dilution does not take place instantaneously.

	Pepsin	Trypsin.
After 2 hours.	Fibrin dissolved.	Still some fibrin.
„ 4 „	„	All dissolved.

II. *Experiment.*

Emulsin 0.1% + free methylhydrazine 0.64%
Pepsin 0.1% + „ „

After 2 hours at 40°C was added:

To Emulsin 0.2g. amygdalin,
To Pepsin 0.2% HCl and 3 flocculi of fibrin.

After 15 Min. at 40°, the tests showed that emulsin was still active in the 0.64% solution of free methylhydrazine, but it was weaker than in the control case.

After one hour at 40°C,

Pepsin { 0.64% free methylhydrazine—Fibrin not dissolved at all, even not after 2 days.
Control: All fibrin dissolved.

III. *Experiment.*

Emulsin 0.1% + free methylhydrazine 0.64%.

Kept at 32°C for 20 hours, and 0.1 grm. of amygdalin added and warmed.

Immediately tested:

Control: —Odor of benzaldehyde.

Treated:—No odor at all.

IV. *Experiment.*

Takadiastase 0.1% + free methylhydrazine 0.32%.

After 20 hours at 24° 10 c.c. of a 2% starch paste were added, the mixture kept at 40° for 2 hours, then evaporated and extracted with alcohol to remove the methylhydrazine. The residue was treated with some water.

Control:—Already after ½ hour, no longer any iodine reaction for starch.

Treated:—Blue starch reaction.

These tests leave no doubt that hydrazine and methylhydrazine injure enzyms very much or kill them.

3) *Hydroxylamine.*

Pepsin 0.1% + free hydroxylamine 1%
Trypsin 0.5% + { free hydroxylamine 0.5% „ „ „
Diastase 0.1% + hydroxylamine 1%
Emulsin 0.1% + „ „

After keeping at 40° for 2 hours was added:

to pepsin: 0.2% HCl and three flocculi of fibrin.
to trypsin: 0.2% Na_2CO_3 and „ „ „
to diastase: 0.1 grm. of starch in the form of starch paste.
to Emulsin: 0.1 grm. amygdalin.

and the mixtures kept at 40°C for 2 hours:

Pepsin: Control:—Fibrin dissolved completely.[1]
1% hydroxylamine:—Fibrin undissolved.
Trypsin: Control:—Almost all fibrin dissolved.
0.5% hydroxylamine:—Fibrin unattacked.
1% „ :— „ „

[1] The fibrin had dissolved already after 30 Min.

Even after 24 hours standing at the ordinary temperature, the fibrin was not dissolved where hydroxylamine had been added.

Diastase: Control:—No starch reaction with iodine.

1% hydroxylamine:—Strong starch reaction.[1]

Emulsin: immediately tested after adding amygdaline.

Control:—Strong odor developed.

1% hydroxylamine:—Slight odor of prussic acid and benzaldehyde.

This test was repeated by adding amygdalin after heating the enzym solution with hydroxylamine to 40° for 4 hours.

Control:—Strong odor.

1% hydroxylamine:—No odor.

These observation on the injurious action of the hydroxylamine on enzyms are in accord with a former observation of *O. Loew* on diastase.[2]

The following table shows the results obtained:

	Pepsin.	Trypsin.	Emulsin.	Diastase.
Free NO_2H	Kills at 0.2% in one hour at 40°C.	Kills at 0.05% in one hour at 40°C.	Kills at 0.05% in 16 hours at 18°.	——
Free N_2H_4	Kills at 1% in two hours at 40°C.	Kills at 1% in two hours at 40°C.	Nearly kills at 0.1% in 8 hours at 40°C.	Kills at 1% in two hours at 40°C.
Free $N_2H_2.CH_3$	Kills at 0.64% in two hours at 40°C.	Injures at 0.032% in one hour at 40°C.	Kills at 0.64% in 20 hours at 32°C.	Kills at 0.32% in 20 hours at 24°C.
Free $NH_2.OH$	Kills at 1% in 2 hours at 40°C.	Kills at 1% in 2 hours at 40°C.	Kills at 1% in 4 hours at 40°C.	Kills at 1% in two hours at 40°.

Conclusion.

1. Enzyms in high dilution are not killed by small quantities of dicyanogen. Hereby another essential difference between the chemical behavior of the living protoplasm and that of enzyms is established.

[1] Small doses of iodine are changed to HI by hydroxylamine, hence an excess must be added here to obtain the starch reaction.

[2] Journ f. prakt. Chem. 1888. p. 104.

2. Nitrous acid in very high dilution is more injurious for enzyms than equally diluted nitric acid.

3. Hydrazine, methylhydrazine and hydroxylamine in dilute neutral solutions destroy the activity of enzyms. This would be best explained if the active grouping in the enzyms are either aldehyde or ketone groups. According to *Loew's* present view, ketone groups alone can come here into consideration.

Ueber fungicide Wirkungen von Pilzculturen.

VON

Y. Kozai und O. Loew.

Es ist seit lange bekannt, dass die Culturen mancher Bacterienarten Stoffe enthalten, welche das Wachstum anderer Bacterienarten hemmen oder verhindern. Bei den Culturen des Bac. pyocyaneus ist es ein Enzym, welches geradezu manche andere Bacterien auflöst.[1] Auch die Entwicklung von Schimmelpilzen ist öfters auf Culturflüssigkeiten nicht möglich, in denen sich gewisse Bacterienarten entwickelt haben, obwohl es sich hier nicht um Aufloesung der Mycelfäden handelt. In neurer Zeit haben ferner *E. Bourquelot* und *Hérissey*[2] beobachtet, dass ein Extract von *Aspergillus niger* die Gärtätigkeit der Hefe beeinträchtigt, die Hefe selbst aber nicht tötete. Diese Wirkung wurde selbst nach dem Aufkochen der Loesung nicht aufgehoben.

Die Beobachtung nun, dass der in Japan unter dem Namen Miso bekannte vegetabilische Käse selbst in der heissesten Zeit des Sommers nicht schimmelt, trotzdem er in hoch feuchtem Zustande dem Staube der Luft ansgesetzt in offnen Läden feil gehalten wird, bewog uns, auch die Culturflüssigkeit des *Aspergillus oryzae* auf fungicide Eigenschaften zu prüfen. Jener Miso wird nämlich mit Hülfe dieses Pilzes, resp. dessen Enzymen aus gekochten Soyabohnen dargestellt.

Er enthält 50-60% Wasser, 5-11% Kochsalz, 6-12% Rohprotein, 5-6.5% Fett und 13-24% Kohlehydrate und Extractstoffe. Die Reaction ist meist ganz schwach sauer. Der Kochsalzgehalt ist zu gering, als

[1] Siehe R. Emmerich und O. Loew. Z. Hyg. 1898.

[2] Jahresber, f. Thierchemie 1895, S. 623.

dass derselbe das Schimmeln verhindern könnte. Wir liessen ihn in einem offnen Becherglase während des Monats August bei einer alltäglich auf 33-35°C steigenden Temperatur stehen und beobachteten dabei eine allmälich sich entwickelnde Hefeschichte, welche dann von *Sarcina* überwuchert wurde. Schliesslich wurde auch diese durch *Bac. prodigiosus* verdrängt. Es wurde keine Spur von *Penicillium* oder *Aspergillus* entwickelt. Die Reaction war schliesslich alkalisch geworden. Um nun zu prüfen ob *Aspergillus oryzae* Stoffe produciren kann, welche auf ihn selbst sowohl als auf nahestehende andere Fadenpilze schädlich wirken, wurde jener Pilz auf folgender Loesung cultivirt:

Pepton — 1%
Zucker — 0.5%
$K_1H_2PO_4$ — 0.2%
$MgSO_4$ — 0.02%

Es wurden 4 Kolben, je 500 cc. dieser Loesung enthaltend, aufgestellt und nach dem Sterilisiren inficirt, am 18 September. Um die Sporenbildung zu verhindern und die Mycelbildung zu begünstigen, wurden die Kolben täglich umgeschüttelt. Einer der Kolben wurde am 6 October geöffnet, der Inhalt (der noch etwas unzersetztes Pepton enthielt) durch sterilisirte Filter in sterile Kolben filtrirt und ein Teil direct, der andere nach dem Aufkochen mit Penicilliumsporen inficirt. Nach einigen Tagen zeigte sich in beiden Flaschen eine sehr langsam sich entwickelnde Schimmelvegetation. Es wurden desshalb die anderen Kolben noch länger stehen gelassen, bis das Mycel den Nährboden erschöpft hatte und dem Absterben unterlag. Das letztre schien uns aus der allmälich eintretenden Schwarzfärbung der Flüssigkeit (Folge von austretenden oxydirenden Enzymen?) zu folgen.

Am 20 November wurden nun zu einem Kolben 2 g sterilisirtes Pepton gesetzt und dann wie oben verfahren. Es ergab sich, dass diesmal selbst nach 11 Tagen bei 10-16°C. keine Spur einer Schimmelvegetation eintrat. Auffällig war, dass auch in der einen Moment aufgekochten Portion sich keine Entwicklung nach der Impfung zeigte.

Es muss also die Production eines gewissen fungiciden Stoffes durch

den Pilz *Aspergillus oryzae* gefolgert werden. Diese Substanz ist aber für verschiedene Pilze nicht in gleichem Maase schädlich.

Im Anhang hiezu wurde noch ein zweiter Versuch mit *Penicillium* sporen gemacht, welche diesesmal auf eine nur schwach alkalisch reagirende Culturflüssigkeit des *Bac. pyocyaneus* ausgesät wurden. Es fand auch nach Wochen keine Entwicklung von *Penicillium* statt, während im Controlversuch dieselbe sehr lebhaft war.

Zur Frage der Existenz des Pyocyanolysins.

VON

O. Loew und Y. Kozai.

Bulloch und *Hunter* hatten vor mehreren Jahren beobachtet, dass die Culturen des Bac. pyocyaneus einen Körper enthalten, welcher Blutkörperchen auflöst. Sie fanden ferner, dass dieser Körper vorzugsweise in den Zellen bleibt, so dass Filtrate der Culturen weit schwächer wirken, als sterilisirte unfiltrirte Culturen. Darauf hin haben wir gesucht, Bedingungen zu finden unter welchen dieser Körper, den *Bulloch* und *Hunter* für ein Enzym hielten und Pyocyanolysin nannten, in besonderem Maase entsteht.[2] Wir beobachteten dabei, dass eine Peptoncultur bei reichlichem Luftzutritt die Eigenschaft Blutkörperchen zu lösen besonders stark zeigte, aber für Mäuse ganz harmlos war, während die Bouilloncultur bei nur geringem Luftzutritt toxische Eigenschaften hatte und in weit geringerem Maase Blut löste. Es war für uns allerdings etwas überraschend, dass ein Blut lösendes Enzym bei subcutaner Injection harmlos für Mäuse sein sollte. Indessen die Beobachtung von *Bulloch* und *Hunter* waren auch von *Weingeroff*,[3] ferner von *Nencki* und *Sieber* gemacht worden,[4] Da ferner B. pyocyaneus besonders reichlich enzymbildend ist[5] war auch die Bildung eines blutlösenden Enzyms nicht

[1] Centralbl. f. Bakt. Band 28, S. 866.

[2] Diese Bulletins, Bd. 4, No. 5.

[3] Centralbl. f. Bakt. Bd. 29, S. 777.

[4] Briefliche Mitteilung von M. Nencki an den einen von uns.

[5] In neurer Zeit constatirte *Eijkmann* (C. Bakt. Bd. *29*, S. 848) die Bildung von Lipase, ferner die eines elastische Fasern lösenden Enzyms (Ibid. Bd. *35*, S. 2), durch den *Bac. pyocyaneus*. Nach *Eijkmann* erkennt man am einfachsten die blutlösenden Eigenschaften mit Blut-Agar. C. Bakt. *29*. S. 847.

unwahrscheinlich, da ferner unsere Culturen nur schwach alkalisch reagirten, suchten wir den Grund der Blutloesung auch nicht im Alkaligehalt, um so weinger als nach *Myers*[1] hinreichend schwaches Ammoniak keine Haemolyse verursacht, wenn die Blutkörperchen in physiologischer Kochsalzloesung suspendirt sind.[2] Immerhin war es uns auffallend, dass die haemolytische Wirkung durch Kochen der Loesung nicht aufgehoben wurde und sich keine giftige Wirkung bei Mäusen constatiren liess.

In neuester Zeit hat nun *Jordan*[3] ebenfalls die haemolytische Wirkung von Pyocyaneuskulturen beobachtet, aber dieselbe lediglich als Folge des Alkaligehalts der Culturen erklärt. Werden die Culturen genau neutralisirt, so bleibt die Haemolyse aus. Wir haben diesen Versuch wiederholt und können diese Beobachtung im Wesentlichen bestätigen.

Als 2 cc. einer 5% Aufschwemmung von Blutkörperchen mit 2 cc. der 15 Minuten auf 60° erwärmten Cultur 24 Stunden im Brutschrank blieben, war Lösung der Blutkörperchen eingetreten; als aber die Cultur vorher genau neutralisirt wurde, war nach 24 Stunden keine Haemolyse eingetreten. Erst nach einen weiteren Tag trat allmälich Loesung ein, doch kann dieses kaum auf eine Enzymwirkung gedeutet werden. Weitere Versuche haben ergeben, dass schon auffallend geringe Mengen von Natriumcarbonat Haemolyse herbeiführen können. Als 2 cc. einer Blutkörperchenaufschwemmung mit 2 cc. einer 0.001% Sodaloesung 2½ Stunden bei 32° gehalten wurden, war schon etwa die Hälfte, nach 20 Stunden alles gelöst. Es ist dieses um so auffallender, als das Blut im lebenden Thier doch auch eine alkalische Reaction besitzt.

[1] C. Bakt. *28*, S. 237.

[2] Erst wenn eine gewisse Menge secundäres Natriumphosphat zugefügt wird, erfolgt Haemolyse.

[3] C. Bakt., Bd. *33*, S. 274.

On the Microbes of the *Nukamiso*.

BY

S. Sawamura.

The name of *Nukamiso* is given in Japan to a preparation, resulting from the spontaneous fermentation of a mixture of rice bran, common salt and water. It is used for softening and rendering palatable certain fruits and roots. According to *Inouye*[1] it has following composition.

Water.	75.6%
Lactic acid.	2.6
Sugar.	3.4
Sodium chlorid.	8.1
Proteids, amidocompounds, fats, mineral matters, starch.	10.4

The most striking chemical change caused by that fermentation is the increased production of sugar and acids. The writer estimated the quantity of acides and sugar in *Nukamiso* fermented by keeping the fresh mixture in a warm (20—25°C) place for 20 hours.[2] The quantity of normal soda solution necessary for neutralizing 100 cc of the filtrate was 5.2 cc, while the acidity of the original rice bran mixture was only 0.4 cc. The quantity of sugar calculated as dextrose was as follows:—

Original mixture	0.041%
Nukamiso.	0.534 „

By preparing a plate-culture of chalk-glucose medium with freshly prepared *Nukamiso* four kinds of bacilli which produced acids were isolated. The microbes have the following properties.

[1] This bulletin Vol. II. No. 4.

[2] The mixture consisted of 100 gr of rice bran, 50 gr of common salt and 1 litre of water.

No. 1.

Form. The cell cultured in bouillon for 24 hours at 37°C is 0,5μ wide and 1—2μ long. Two are generally united; they are motile by peritric flagella. Spore formation is not observed.

Staining. Gram's method negative.

Oxygen. Aërobic.

Bouillon. A feeble scum and a little deposit are formed.

Gelatin plate-culture. A round, light, yellow, moist, bright, sharply defined colony. An elevated point is observed in the centre. It grows to a moderate size. Deep colony appears as a white point.

Gelatin streak-culture. A light yellow, moist, homogeneous colony. Gelatin is not liquefied.

Gelatin stab-culture. Thread-like growth to the bottom.

Agar streak-culture. A white, homogeneous colony, condensed water clear.

Potato culture. An elevated, moist, at first gray but afterwards yellow colony.

Milk culture. It is coagulated with an acid reaction.

Gas. It is evolved in glucose-bouillon. The gas consists of CO_2 and H.

Indol reaction. Positive.

Chemical activity. It does not saccharify starch. Acid produced in a mixture of 10 gr of rice bran and 100 cc of water for 3 days at 36°C was 0.780% calculated as lactic acid from the quantity of normal soda solution necessary for neutralisation.

No. 2.

Form. The cell is 0.6μ wide and 2—4μ long, and has rounded ends. It is motile and flagella seem to grow in one end of the rod.

Gram's method. Positive.

Oxygen. Aërobic.

Bouillon. A feeble scum and a moderate deposit are produced, but in glucose-pepton water a thick scum which finally breaks.

Gelatin plate-culture. A round, sharply defined, somewhat transparent,

homogeneous, moist colony, which never grows larger than 2 mm in diameter.

Gelatin streak-culture. A light yellow, moist, homogeneous colony. Gelatin is not liquefied.

Gelatin stab-culture. Thread-like growth to the bottom.

Agar plate-culture. A round, elevated, somewhat transparent, moist colony which does not grow larger. By weak magnification it is the same. Deep colony is a white point.

Agar streak-culture. A yellowish white, moist, bright, homogeneous colony.

Potato culture. A dark yellow, moist, bright, homogeneous colony,

Milk culture. It is coagulated with an acid reaction.

Gas. It is not evolved in glucose-bouillon.

Indol reaction. Negative.

Chemical activity. Starch is not saccharified, and sugar is not formed also in *Nukamiso* by this bacillus. Acid produced in a mixture of 10 gr of bran and 100 cc of water for 3 days at 36°C was 1.327% calculated as lactic acid, and that produced in glucose-buillon containing some Ca CO_3 in 3 days at 36°C was 1.007% of lactic acid calculated from CaO dissolved. The acid produced was found to be lactic acid by examining the properties of the zinc salt.[1]

Some alcohol is formed from glucose, which was confirmed by the formation of iodoform.

Since the already known lactic ferments such as Bacillus acidi lactici *Hueppe*, Bacterium acidi lactici *Grotenfeldt* and *Kozai's* bacilli are all not motile this microbe seems to be a new species.

No. 3.

Form. The cell is 0.4μ wide and 1μ long, and it is motile by peritric flagella.

[1] The nutritive solution of glucose with $CaCO_3$, in which this bacillus was cultured, was filtered. The filtrate, after having been acidified with P_2O_5, was evaporated to dryness. The residue was treated with either and filtered. The filtrate was evaporated but no crystal was formed, the residue being a syrupy mass. It was neutralised with Zn CO_3, and by examining the crystal-form of the zinc salt and its behavior towards alcoholic ammonia, it was proved to be zinc lactate.

Two are usually united, and spore-formation is not observed.

Gram's method. Negative.

Oxygen. Aërobic.

Bouillon. It becomes turbid, but no scum is formed.

Gelatin plate-culture. A round, elevated, sharply defined, white, moist, bright, homogeneous colony. By weak magnification it is the same. Deep colony is a white point.

Gelatin streak-culture. A white, moist, bright, homogeneous colony. Gelatin is not liquefied.

Gelatin stab-culture. Thread-like growth to the bottom.

Agar streak-culture. A white, moist, bright, flat colony.

Milk culture. It is coagulated with an acid reaction.

Potato culture. A gray moist, elevated colony. Gas bubbles are formed on the colony.

Gas. Gas consisting of CO_2 and H is vigorously produced in glucose bouillon.

Indol reaction. Negative.

Chemical activity. It does not saccharify starch. Acid produced in *Nukamiso* above described for 3 days at 36°C was 0.654% calculated as lactic acid and that produced in glucose bouillon containing $CaCO_3$ in 3 days at 36°C was 0.453% of lactic acid calculated from dissolved CaO. This microbe can produce the characteristic smell of *Nukamiso*.

This is probably Bacillus chologenes *Kruse*, which resembles very much the coli-bacillus.

No. 4.

Form. The cell is 0.5—0.7μ wide and 2—4μ long and is motile by peritric flagella. Spore-formation is not observed.

Gram's method, positive.

Oxygen. Aërobic.

Bouillon. A stick scum is formed, the medium remaining quite clear.

Gelatin plate-culture. A round, flat, gray, roughly defined colony which grows very large.

Gelatin streak-culture. It liquefies quickly gelatin.

Gelatin stab-culture. Thread-like growth to the bottom, the surface being quickly liquefied.

Agar streak-culture. A light brown, folded, characteristic colony.

Milk culture. It is coagulated with an acid reaction.

Potato culture. Characteristic folded colony which is at first faintly red, and changes to gray afterwards.

Gas. It is not evolved.

Indol reaction. Positive.

Chemical activity. It saccharifies starch, and produces 0.296% of glucose in a mixture of 10 gr of bran and 100 cc of water for 24 hours at 36°C, and acid produced in the same mixture for 3 days at 36°C was 0.770% calculated as lactic acid. A feeble red tint is produced in *Nukamiso*.

By these properties this microbe is regarded as Bacillus mesentericus ruber *Globig*.

From an old *Nakamiso* the writer isolated a bacillus which was identified to be Bacillus mesentericus vulgatus *Flügge* and a kind of Kahm-yeast, which produces a weak alcoholic fermentation on a nutritive solution containing glucose.

In order to see which microbe produces most acid they were inoculated into a sterilized *Nukamiso* and kept for 24 hours at 36°C. The quantity of normal soda necessary to neutralize 100 cc of the filtrate from the above culture was as follows:—

No. 1.	12cc
No. 2.	20 „
No. 3.	10 „
No. 4.	12 „
No. 2.+No. 4.	22.5 „
No. 1.+No. 2.+No. 3.+No. 4.	25.0 „

From these figures it is clear that the chief acid producer is Bacillus No. 2, but the symbiosis with the other microbes increases the production of acid. The smell characteristic for *Nukamiso* is probably produced by a Mesentericus species, and the organism No. 3. The production of sugar in *Nukamiso* is solely due to the activity of the Mesentericus species.

The species of saccharomyces present does not participate in the fermentation, since its fermentative power is nearly nil. The writer observed in the case of saccharification of mannan by Bac. mes. vulgatus, the acceralating action of a certain wild yeast on this bacillus.[1] In order to see whether the function of this wild yeast be analogous to it the mixture of Bac. mes. vulgatus with the saccharomyces was cultured for 3 days at 20°C in glucose-bouillon containing $CaCO_3$ and the quantity of CaO dissolved by the acid formed was determined.

Control. 0.618%

Bac. mes. vulgatus + the Saccharomyces. 0.910 „

In the second experiment a mixed culture of Bac. mes. vulgatus and that yeast were left to act upon starch, 2% of which were suspended in bouillon. The sugar formed in one week at the ordinary temperature was as follows:—

Control. 0.79%

Bac. mes. vulgatus + the wild yeast. ... 0.82 „

From these figures it follows that the saccharomyces has indeed some accelerating effect upon the action of the bacteria.

We may conclude as follows:—

I. By the fermentation of *Nukamiso* sugar and acids are formed in a moderate quantity.

II. In fermented *Nukamiso* there are present various microbes, of which the writer isolated four kinds of bacilli and a Saccharomyces.

III Sugar is produced exclusively by a Mesentericus species.

IV. Several microbes that can produce acid are present in *Nukamiso*, but the chief acid-producer is a bacillus, which seems to be a new species.

V. The aroma characteristic for *Nukamiso* seems chiefly to be produced by a Mesentericus species.

VI. The Saccharomyces present in *Nukamiso* seems to have no other effect than to acceralate somewhat the bacterial actions.

[1] This bulletin Vol. V No 2.

Ueber den Kalkgehalt verschiedener tierischer Organe.

VON

M. Toyonaga.

Das Muskelfleisch hat nach mehreren Autoren einen höheren Gehalt an Magnesia als an Kalk, was wahrscheinlich mit der relativ geringen Zellkernmasse zusammenhängt.[1] Es zeigt sich jedoch ein Unterschied zwischen dem Kalkgehalt der Muskeln von Batrachiern und Fischen einerseits, und demjenigen der Muskeln der Warmblüter anderseits; so ergiebt sich im Durchschnitt für 1000 Teile frischen Muskels von Warmblütern 0,0954 Teile Calcium, bei Kaltblütern aber 0,2913 Teile Calcium.

Ferner ist das Verhältniss $\frac{Ca}{Mg}$ der Muskelgewebe beider Tiergruppen sehr verschieden.

Muskel der Kaltblüter.		Muskel der Warmblüter.[2]
$\frac{Ca}{Mg}$	1.26	0.34

Von einigem Interesse schien es nun, die glatten mit den quergestreiften Muskeln in dieser Hinsicht zu vergleichen, da die glatten Muskeln eine niedere Entwickelungsstufe des Muskelgewebes darstellen, und die relative Grösse der Zellkerne verschieden ist. Vergleichende chemische Untersuchungen beider Muskelarten sind nur spärlich vorhanden.

[1] Vergleiche meine früheren Abhandlungen in diesen Bulletins, Band 5.

[2] Durchschnitt aus mehreren Bestimmungen von Muskeln verschiedener Tiere nach *Katz*.

Von Interesse ist die Beobachtung Vincents,[1] dass die glatten Muskeln 6-8 mal so viel Nucleoproteid enthalten als die quergestreiften, und dass der Herzmuskel einen Uebergang zwischen beiden bildet. Beide Muskelarten geben ein Salzplasma, welches entweder von selbst gerinnt oder durch Verdünnung. Ob das Mehr von Nucleoproteid in den glatten Muskeln gänzlich dem relativ grösserem Zellkern der glatten Muskelfasern zuzuschreiben ist, wäre noch zu untersuchen. Immerhin schien es von Interesse, das Verhältniss $\frac{Ca}{Mg}$ in beiden Muskelarten von demselben Tiere zu bestimmen. Ich wählte die Schenkelmuskeln des Pferdes und verglich sie mit den Bauchmuskeln. Leider stellen die letzteren Muskeln keineswegs nur ein Gewebe aus glatten Muskelfasern dar, sondern enthalten noch quergestreifte Muskelfasern und Bindegewebe. Es können daher die erhaltenen Zahlen nur annähernde Werte für die glatten Muskelfasern sein. Ich verfuhr im Wesentlichen nach der in meinen früheren Arbeiten (l. c.) erwähnten Methode und erhielt die folgenden Resultate.

In 1000 Teilen frischer Substanz sind enthalten:

	CaO	MgO	$\frac{Ca}{Mg}$
Quergestreifter Muskel des Pferdes.	0,064	0,322	$\frac{0.24}{1}$
Glatter Muskel des Pferdes.	0,07	0,292	$\frac{0.29}{1}$

Man erkennt hieraus, dass in der Tat die glatten Muskeln, obgleich noch gemischt mit quergestreiften, einen etwas höheren Kalkgehalt ergeben als die quergestreiften.

[1] Zeitschrift f. physiolog. Chemie. Band 34, S. 417, (1902).

Hodensubstanz.

Obgleich die Hoden drüsige Organe sind, so erscheint doch die Zellkernmasse derselben geringer als die der Leber oder Pancreasdrüse. Kalk- und Magnesiagehalt der Drüsen scheint manchmal, vielleicht unter pathologischen Einflüssen, abnormen Schwankungen zu unterliegen, jedoch nicht in dem Sinne, dass der Magnesia-gehalt über den Kalkgehalt steigt, sondern dass der Kalkgehalt enorm zunimmt und der Magnesiagehalt abnimmt. So fand *Lüning*[1] in der Asche der Pancreasdrüsen von zwei krebskranken Frauen das eine Mal (a) Ca=2,55% und Mg=1,48%, das andere Mal (b) Ca=16,94% und Mg=0,37%.[2] Die Aschenprocente der frischen Drüsen waren nahezu gleich, nämlich 1,04 und 1,02 resp.

Auf 1000 Teile frischer Substanz berechnet würde sich ergeben:

(a)	(b)
Ca—0.2663	1.7356
Mg—0.1541	0.0383

Dieses abnorme Sinken des Magnesiumgehalts im Falle (b) beim Pancreas, erinnert an einen ganz ähnlichen Fall, beobachtet an der Leber, von *Oidtmann.*

Dieser[3] fand (1858) in der Leber 1.1% Asche und in dieser Asche= 3.62% CaO und nur 0.19% MgO, oder 0.289 Teile Ca und 0.017 Teile Mg auf 1000 Teile frischer Substanz; es ist also in diesen abnormen Fällen:

	Pancreas (b).	Leber (nach Oidtmann).
$\frac{Ca}{Mg}$ =	45.3	17.0

[1] Die anorganischen Bestandteile des Pancreas, Würzburg 1899.

[2] Die eine Frau (a) litt an Magenkrebs, die andere (b) an Eierstockkrebs.

[3] In der Asche der Milz fand dieser Autor auf 7,48% Kalk nur 0.49% Magnesia; es ist also hier das Verh. $\frac{Ca}{Mg}$ =18.1

Sonst bewegt bei Drüsen sich dieses Verhältniss $\frac{Ca}{Mg}$ zwischen 1 und 6.

Aus Hoden von Fischen wurden bekanntlich interessante Substanzen gewonnen, die Protamine, aber es existirt doch noch keine vollständige quantitative Analyse des Hodens von Säugetieren,[1] woraus wir die Menge Albumim, Globulin, Nucleoprotein, Mucin, Lecithin, etc. entnehmen könnten. Sogar die Trockensubstanz ist nicht genau bestimmt worden. *Miescher* giebt zwar an, dass der Wassergehalt 75 procent und die Trockensubstanz 25 procent betrage; wahrscheinlich hatte er aber den Hoden gemischt mit Bindegewebe der Bestimmung unterworfen. Bei meiner Bestimmung entfernte ich das Bindegewebe so sorgfältig wie möglich und fand dann den Wassergehalt weit bedeutender, nämlich 85.39%. Ich analysirte die Hodensubstanz des Pferdes und des Stieres[2] mit folgendem Resultat:

	In 1000 Teilen.			
	Total Asche.	CaO	MgO	$\frac{Ca}{Mg}$
Pferdehoden	9.550	0.096	0.256	$\frac{0.45}{1}$
Stierhoden a)	9.943	0.102	0.214	$\frac{0.51}{1}$
b)	10.109	0.091	0.237	

[1] Vor Kurzem hat *Levene* aus Rinderhoden eine Nucleinsäure dargestellt, welche bei Spaltung unter andern Guanin, Thymin und Cytosin lieferte.

[2] Den Chlorgehalt der Stierhodenasche fand ich zu 0.418%. Bei der Pancreasasche beträgt derselbe 2.5–2.6%.

Beim Vergleich der Trockensubstanzen ergiebt sich:

	Quergestreifter Muskel des Pferdes.	Milchdrüse des Rindes.	Hoden des Pferdes.
CaO	0.0323%	0.2517%	0.668%
MgO	0.1619%	0.0639%	0.1773%
$\frac{Ca}{Mg}$	0.24	4.67	0.45[1]

Wir finden daher, dass der Kalkgehalt des Hodens geringer ist als der von Pancreas, Milz und Leber, dass aber andererseits das Verhältniss $\frac{Ca}{Mg}$ ein weiteres ist als bei den Muskeln der Warmblüter. Das Secret des Hodens ist aber sehr kalkreich, in Uebereinstimmung mit dem ansehnlichen Gehalt an Zellkernen (Spermatozoen). Es enthält nach *Z. Slowzoff*[2] frisches Menschensperma=0,90% Asche, ferner 9.68% Trockensubstanz und 0.199% Nucleïn (2% des trocknen Spermas). Der Kalkgehalt in der Asche von zwei Alkoholfraktionen des Spermas betrug 22,40% und 15,08%.

Analytische Belege.

	Frische Substanz.	Wasser.	Asche.	$CaCO_3$ (in der Hälfte).	$Mg_2P_2O_7$ (in der Hälfte).
Quergestreifter Muskel.	233.6916 g.	187.243 g.	2.032 g.	0.0134 g.	0.1045 g.
Glatter Muskel.	231.0862 „	187.1316 „	2.1236 „	0.0147 „	0.0938 „
Hodes des Pferdes.	120.6913 „	103.3148 „	1.1521 „	0.0103 „	0.0427 „
Hoden des Stiers (a)	201.5540 „	175.0597 „	2.004 „	0.0184 „	0.0609 „
(b)	186.8451 „	161.9256 „	1.9196 „	0.0152 „	0.0612 „

[1] Das Verhältniss $\frac{Ca}{Mg}$ in der Lunge nähert sich mehr dem in den Hoden als dem in der Leber und Milchdrüse. Schmidt fand in 1000 Teilen Lunge (Mensch) 1.9 CaO und 1.9 MgO, also $\frac{Ca}{Mg} = \frac{1.18}{1}$.

[2] Zeitschrift f. physiolog. Chemie, 35, p. 358.

Behufs einer Uebersicht über die bisher in Bezug auf den Kalkgehalt tierischer Organe erhaltenen Resultate lasse ich folgende Zusammenstellung folgen.

In 1000 Teilen frischer Substanz sind enthalten Calcium:

Muskel des Warmblüter	0.057 (Bunge, Mittel).
„ „ „	0.095 (Katz [1896], Mittel).
Quergestreifter Muskel (Pferd)	0.046 (Toyonaga [1902]).
Glatter Muskel (Pferd)	0.050 „
Weisse Hirnsubstanz (Kalb)	0.041 „
„ „ (Pferd)	0.037 „
Graue Hirnsubstanz (Kalb)	0.263 „
„ „ (Pferd)	0.778 „
Milchdrüse (Kuh)	0.600 „
Hoden (Pferd)	0.069 „
„ (Stier)	0.069 „
Leber (Mensch)	0.284 (Oidtmann).
Periphere Nerven (Pferd)	0.568 (Toyonaga).

Es zeigt sich ferner, dass mit Zunahme des Kalks in drüsigen Organen die Zunahme der Magnesia keineswegs gleichen Schritt hält, sondern in manchen Fallen sogar sehr gering bleibt. Vergleichen wir das Verhältniss Ca/Mg beim Muskel und der weissen Hirnsubstanz mit dem in drüsigen Organen und der grauen Hirnsubstanz, so ergiebt sich:

	Ca/Mg.
Muskel der Warmblüter	0.34 (Katz, Mittel).
Quergestreifter Muskel (Pferd)	0.24 (Toyonaga).
Glatter Muskel (Pferd)	0.29 „
Weisse Hirnsubstanz (Pferd)	0.30 „
„ „ (Kalb)	1.14 „
Graue Hirnsubstanz (Pferd)	2.80 „
„ „ (Kalb)	1.72 „
Milchdrüse (Rind)	4 69 „
Niere	1.84 (Aloy).
„ (Rind)	2.98 (Gossmann).

	Ca/Mg.
Niere (Mensch)	4.25 (Gossmann).
Milz (Rind)	2.52 (Ribaut, Mittel).
„ „	2.79 (Aloy).
Milzpulpa (Rind)	2.70 (Ribaut).
Bindegewebe der Milz (Rind)	3.45 „
Pankreas (Mensch)	1.73 (Lüning 1900).
„ „	4.75 (Gossmann).
Lunge	1.20 (Schmidt).
„ (Pferd)	1.36 (Toyonaga).
Hoden (Stier)	0.51 „
„ (Pferd)	0.45 „
Periphere Nerven (Pferd)	1.56 „

On the Influence of Different Ratios of Lime to Magnesia on the Growth of Rice.

BY

K. Aso.

A number of experiments to find the most favorable ratio of lime to magnesia for plant-growth have been made at this Institute, under Prof. Loew. Furuta studied in this regard the behavior of buckwheat, oats and cabbage in soil culture and the writer[1] that of barley, soy-bean and onion in water culture, and of the mulberry-tree in water as well as in soil culture. Recently, Katayama[2] has also carried out several sand and soil cultures with onion, oats and buckwheat on this line. In all those cases, it became evident that the maximum yield depended, other things being equal, upon a distinct ratio of lime to magnesia and that the best ratio is not the same with every kind of crops.

Since rice-culture is a most important factor[3] in agriculture of Japan, it occurred to me that this principle should be also studied with the rice crop. The soils differ widely in chemical composition and the farmer never knows whether liming would be in order or not. Generally, however, the farmers of Japan apply too much lime and the injuries thus produced have induced the local government of Kiushiu to issue a law prohibiting the use of lime. Besides the depression of the harvest also a greater brittleness of straw and grains and a relative decrease of protein result from excessive liming.[4] The content of lime and magnesia in the straw and grains of rice plants are the following in an average:

[1] Bull. College of Agric. Tokyo. Vol. IV. No. 5 and Vol. No. 4.

[2] This Bulletin. p. 102.

[3] The annual production of rice in Japan (without Formosa) amounts in average to 41 millions Koku(=7.4 Mill. Liter.) while the importation amounts to a value of at least 5 million yen annually.

[4] Bull. College of Agric. Tokyō. Vol. I. No. 9.

In 100 parts of air dry matter :

		CaO	MgO	CaO : MgO
Paddy rice	straw	0.26	0.19	1.4 : 1
	not whitened grains	0.03	0.09	1. : 3
Upland rice	straw	0.31	0.24	1.3 : 1
	not whitened grains	0.02	0.07	1. : 3.5

It will be seen from these figures, that the ratio of lime to magnesia in rice is smaller than that in many other plants.

My experiment was carried out as follows :—

Seven *Wagner's* porcelain pots were filled with 7 kilo of airdry sifted soil taken from a paddy field which had not been cultivated for several years. The quantity of available lime and magnesia in this soil was determined by extracting the soil with cold 10 % hydrochloric acid for 48 hours with the following result :

In 100 parts of dry soil ;

CaO	0.70
MgO	0.60

The ratio of lime magnesia was now changed in six pots by mixing the soil with calcium carbonate or pure magnesite[1] (finely powdered) to reach the following ratios : —

Pots.	Quantity of Calcium carbonate added. gr.	Quantity of Magnesite added. gr.	CaO : MgO
a	166.24	0	5 : 1
b	122.86	0	4 : 1
c	79.46	0	3 : 1
d	36.07	0	2 : 1
e (original)	0	0	1 : 1 (nearly)
f	0	68.3	1 : 2
g	0	127.4	1 : 3

[1] This mineral was imported from Germany and contained only minute quantities of lime.

As general manure for each pot served :

Ammonium sulphate	15 grm.
Sodium phosphate	15 grm.
Potassium carbonate[1]	10 grm.

On July 13, the young rice plants[2] (about 36 cm. long) were transplanted from the seed bed. Each pot received three bundles. One bundle was made up of three individuals of equal size. Although this experiment was carried out in a glass house, the treatment was the same as in the field. Towards the end of August the difference in plant growth became very marked, the plants in e showed the best growth of all. These plants also flowered first (Sept. 9.). On September 18, all plants were in flower and on that day a photograph was taken which is reproduced on plate X and which exhibits the difference in development very well. It might be surmised that some ammonia of the sulfate was transformed into carbonate by the potassium or calcium carbonate added and volatilized; and this loss of some nitrogen might have something to do with the difference in growth. But it must be remembered that not only was the dose of nitrogen a very heavy one (ratio: 600 kilo N per ha), and that much more nitrogen was present than could be possibly utilized, but also that the soil was so rich in humus (11%) that a considerable absorption of ammonia was undisputable. Neverthless further experiments are contemplated with such a modification that even a small loss of nitrogen with be practically excluded,[3] viz. P_2O_5 will be applied as superphosphate and K_2O as sulfate.

On November 6, the crop was harvested and left to become airdry. The weight was as follows:

[1] This was applied separately, later.

[2] The variety name was Satsuma.

[3] *P. Wagner* has calculated from many trials with oats, that in average 1 gram of ammonica nitrogen can yield 56.97g. straw+41.33g. of grains, while 1 gram of nitrate nitrogen, 57.03g. straw+42.53g. of grains. From these data it may be seen, that there was a considerable excess of nitrogen in my manured soil compared with the harvest of rice, which in all probability would show not a very different behavior from the related oats.

Pots.	$\frac{CaO}{MgO}$	Full grains.	Empty grains.	Straw.	Total.
a	$\frac{5}{1}$	gr. 20.5	gr. 2.0	gr. 53.5	gr. 76.9
b	$\frac{4}{1}$	30.5	1.5	59.5	91.5
c	$\frac{3}{1}$	44.0	2.0	65.5	111.5
d	$\frac{2}{1}$	58.5	3.5	96.0	158.0
e	$\frac{1}{1}$	98.5	6.5	125.0	230.0
f	$\frac{1}{2}$	84.0	3.0	95.5	182.5
g	$\frac{1}{3}$	79.0	4.0	106.0	189.0

It will be observed from these figures, 1) the lime factor[1] for rice agrees nearly with that of other Gramineae which is between 1 and 2; 2) the rice plant seems to possess a relatively considerable resistance power against an excess of magnesium carbonate[2] since this does not depress the yield so much as the same excess of lime; 3) Rice-culture demands special attention to the proper ratio of lime to magnesia, since the maximal yield depends to a great degree upon the ratio 1 : 1.

These facts induced me to examine the ratio of lime and magnesia in various soils of Japan. The analyses of soils were kindly furnished by Dr. *Tsuneto* and his colleagues of the Geological Survey of the Japanese Empire, who have published a compendious volume on the composition of soils in Japan. These analyses were made in the usual style and do therefore not exactly distinguish between the readily assimilable amounts and the total amounts of lime and magnesia soluble in hot concentrated hydrochloric acid. In examining the long list of analyses I have observed numerous cases in which the ratio $\frac{CaO}{MgO}$ would

[1] Bull. College of Agric. Tokyo. Vol. IV. No. 5.

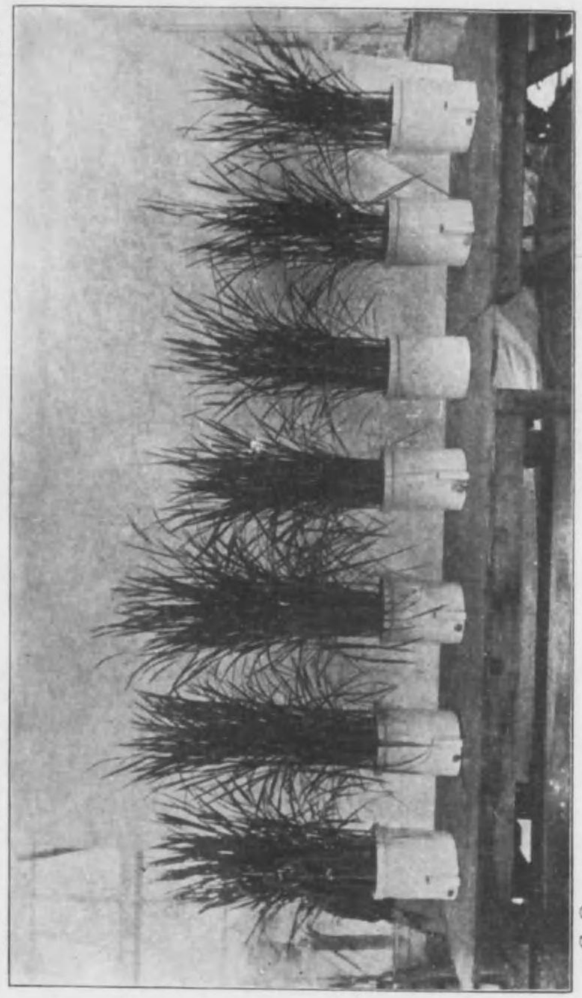
[2] At least in presence of manure of alkaline reaction; an acid reaction of the manure would probably charge this behavior.

not be favorable for a maximal yield of rice. Many of these unfavorable cases are mentioned in the following table:

Locality.	Mother Rock.	In 100 parts of fine dry soil.	
		CaO	MgO
Kurosakamura, Hinogōri, Hoki.	Granite.	0.376	0.986
Kitayamatomura, Ikumagōri, Yamato.	Granite.	0.308	0.888
Mikagemachi, Mukogōri, Settsu.	Granite.	0.058	0.488
Nishinemura, Igugōri, Iwaki.	Granite.	0.412	1.913
Nakanomura, Iishigōri, Idzumo.	Granite.	trace.	0.475
Nagayasumura, Nakagōri, Iwami.	Porphyrite.	0.946	0.158
Nishinamura, Kamogōri, Idsu.	Diabase.	1.618	6.307
Dōshimamura, Kawarumagōri, Iwasyiro.	Andesitic tuff.	0.130	0.004
Daikonjima, Yatsukagōri, Idsumo.	Basalt.	1.259	0.368
Tsudamura, Yatsukagōri, Idsumo.	Basalt.	0.295	0.923
Inomura, Nakagōri, Iwami.	Basalt.	0.180	0.812
Hasamamura, Ōitagōri, Buzen.	Volcanic rock.	1.445	0.312
Fujinemura, Fujigōri, Suruga.	Lava and Lapilli.	0.389	1.784
Kanaokamura, Suntōgun, Suruga.	Volcanic Rock.	0.450	1.735
Tatsukawamura, Iwatagun, Tōtōmi.	Crystalline Schist.	1.440	5.890
Onishimachi, Tanogōri, Kōtsuke.	Crystalline Schist.	0.778	2.189
Kitamura, Suchigōri, Tōtōmi.	Chichibu-paleozoic.	0.612	3.236
Komamura, Irumagōri, Musashi.	Chichibu-paleozoic.	1.610	0.480
Meijimura, Minamiumibegori, Bungo.	Chichibu-paleozoic.	0.575	0.112
Kamisannomiya-mura, Yamagōri, Iwashiro.	Tertiary tuff.	0.391	0.019
Ogimura, Shusugōri, Noto.	Tertiary tufficious sandstone.	1.100	0.407
Ōtamura, Chichibugōri, Musashi.	Tertiary Shale.	0.330	0.940

Locality.	Mother Rock.	In 100 parts of fine dry soil.		Locality.	Mother Rock.	In 100 parts of fine dry soil.	
		CaO	MgO			CaO	MgO
Takidamura, Awagun, Awa.	Tertiary tuff.	0.567	0.033	Gyotokumachi, Higashikatsushikagori, Shimôsa.	Alluvial.	1.545	0.021
Nissakamura, Ogasagôri, Tôtômi.	Tertiary Sandstone.	0.531	2.172	Nikaidômura, Yamabegôri, Yamato.	Alluvial.	0.739	0.200
Tokachihara, Hokkaido.	Diluvial.	2.129	0.400	Hiraidsumimura, Nishiiwaigôri, Rikuchû.	Alluvial.	0.904	0.130
Yoshiminehara, Iwasegôri, Iwashiro.	Diluvial.	1.516	0.096	Ôsumura, Shidagun, Suruga.	Alluvial.	0.120	1.670
Kikyôgahara, Higashichikumagun, Shinano.	Diluvial.	0.448	1.423	Yoshiwaramachi, Fujigun, Suruga.	Alluvial.	0.146	1.602
Kugamura, Katorigun, Shimôsa.	Diluvial.	0.706	0.004	Sekimura, Nagôgori, Kadsusa.	Alluvial.	0.867	0.029
Ômagarimura, Senhokugun, Ugo.	Alluvial.	0.351	1.616	Miomura, Abegôri, Suruga.	Alluvial.	0.272	1.428
Nakazatomura, Takatagun, Idsu.	Alluvial.	2.075	0.461				

PLATE X.



This plate shows the influence of different ratios of lime and magnesia upon rice.

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On the Determination of the Available Amounts of Lime and Magnesia in the Soil.

BY

T. Katayama.

It has been shown by experiments of O. Loew and W. May in Washington[1] and of K. Aso and T. Furuta[2] in Tokyo that the best development of a plant depends, other things being equal, upon a certain ratio of the amounts of lime to the amount of magnesia available to the roots.

In order to reach a maximum harvest, it is necessary to determine the *available* amounts of lime and magnesia in the soil, and then to provide for the proper ratio between these two on the basis of the result obtained, by the addition of the calculated amounts of lime and magnesia compounds.

The extraction of the soil with hot concentrated hydrochloric acid yields very probably more lime and magnesia than can be dissolved by the roots, while the proposed extraction with ammonium chlorid will yield in most cases certainly too small numbers.[3]

Also, the size of the particles to be separated from the soil, previously to the treatment with hydrochloric acid, forms a very important question. Some authors separate all particles smaller than 0.5mm. diameter and treat that fraction with hydrochloric acid of 10% or also of 30% at the ordinary temperature, while others apply boiling heat. Furuta separated all particles smaller than 0.25mm. and treated this fine sand+silt+clay'

[1] Bul. No I, Bureau of Plant Industry Washington 1901.

[2] Bul. of the College of Agriculture, University of Tokyo, vol IV, No. 5.

[3] *Immendorf* proposed recently boiling with $\frac{1}{5}$ normal sulphuric acid for 30 minutes and to determine in this solution the easily available lime and magnesia.

with hot conc. hydrochloric acid. I have tried the following modification:

I. The fine earth, <0.25m.m was treated with hydrochloric acid of 5% at the ordinary temperature, in the proportion of 25g : 75c.c, for 24 hours.

II. The fine earth, <0.25m.m was treated with hydrochloric acid of 10% at boiling temperature for 50 minutes, in the proportion of 25g : 50c.c.[1]

The determination by the former method yielded, however, too small a number for magnesia, leading to a ratio $\frac{CaO}{MgO}$ which would differ too much from the observations of *Aso* and *Furuta* made a year previous with water and soil cultures.[1]

Hence only the second method was adhered to. The percentages of lime and magnesia thus obtained served as a basis for the calculations. In order to observe whether different soils would give equally reliable results with the same method, I have compared the sandy soil from an orchard near Kawasaki, about 15 miles south-west from Tokyo, with a loamy soil, from Komaba, a suburb of Tokyo, the same soil which had served *T. Furuta* for his trials. Both soils were examined in the air dry state.

The analysis gave the following data:

	Lime		Magnesia	
Fine earth of the soil of Kawasaki = 68.8%	{0.615% / 0.650 „}	average 0.63%	{0.823% / 0.786 „}	average 0.80%
Fine earth of the soil of Komaba = 76.33%	{0.597% / 0.587 „ / 0.603 „}	average 0.60%	{0.486% / 0.516 „ / 0.474 „}	average 0.49%

The pots for the cultures with the Kawasaki soil held 5 kilo, which amount of soil contained therefore 21.7g Ca O and 27.5g Mg O. In order to procure the desired ratios the following additions were necessary:

[1] After this treatment with HCl. 200c.c of water was added and heated again to boiling for 10 minutes, then left standing for 15 hours before the filtration.

[2] *Söderbaum* observed recently that a hydrochloric acid of 2% extracted in 48 hours at the ordinary temperature only a part of the available plant nutrients.

I	$\frac{Ca\,O}{Mg\,O}=\frac{0.75}{1}$	No addition.
II.	$\frac{Ca\,O}{Mg\,O}=\frac{2}{1}$	33.3g Ca O = 59.4g $Ca\,CO_3$
III.	$\frac{Ca\,O}{Mg\,O}=\frac{3}{1}$	60.8g Ca O = 108.6g $Ca\,CO_3$
IV.	$\frac{Ca\,O}{Mg\,O}=\frac{4}{1}$	88.3g Ca O = 157.7g $Ca\,CO_3$

The pots for the cultures with the soil from Komaba[1] held 4 Kilo, which amount of soil contained therefore 18.3g Ca O and 14.9g Mg O. Hence the following additions were here required:

I.	$\frac{Ca\,O}{Mg\,O}=\frac{1.2}{1}$	No addition.
II.	$\frac{Ca\,O}{Mg\,O}=\frac{2}{1}$	11.5g Ca O = 21.8g $Ca\,CO_3$
III.	$\frac{Ca\,O}{Mg\,O}=\frac{3}{1}$	26.2g Ca O = 46.9g $Ca\,CO_3$
IV.	$\frac{Ca\,O}{Mg\,O}=\frac{4}{1}$	41.3g Ca O = 73.6g $Ca\,CO_3$

Now if my determination of lime and magnesia in the soils corresponds really to the available amounts, the results obtained with those soils limed in certain degrees, must agree with those obtained with cultures in quartz-sand or water, in which the lime and magnesia were present in the same ratios and in form of soluble salts. The soils were manured in the ratio of 10g of monopotassium phosphate, 6.3g potassium sulfate, 10g sodium nitrate for 5 Kilo.

Sand culture.

About 15 Kilo of pure quartzsand were left with conc. hydrochloric acid for 3 days, and then well washed with water, until every trace of acid reaction had disappeared.

Five flower pots received each 700 grms of this sand, and 14g of

[1] This soil from a depth of one foot was analysed some years before by *T. Furuta*, while for my analysis and cultures served the surface soil only, hence some small discrepancies are easily accounted or.

precipitated air dry aluminium silicate (2% of the sand) to increase the water holding capacity.

The solutions applied had the following composition:

General Manure: 0.1‰ KCl, 0.3‰ $K NO_3$, 0.2‰ $K_1H_2PO_4$, trace $Fe SO_4$

(1)	$\frac{Ca O}{Mg O}=\frac{1}{1}$	0.3‰ Ca O = 0.879‰ $Ca (NO_3)_2$ 0.3 „ Mg O = 0.895 „ $Mg SO_4$
(2)	$\frac{Ca O}{Mg O}=\frac{2}{1}$	0.4‰ Ca O = 1.117‰ $Ca (NO_3)_2$ 0.2 „ Mg O = 0.596 „ $Mg SO_4$
(3)	$\frac{Ca O}{Mg O}=\frac{3}{1}$	0.45‰ Ca O = 1.318‰ $Ca (NO_3)_2$ 0.15 „ Mg O = 0.447 „ $Mg SO_4$
(4)	$\frac{Ca O}{Mg O}=\frac{4}{1}$	0.48‰ Ca O = 1.406 „ $Ca (NO_3)_2$ 0.12 „ Mg O = 0.358 „ $Mg SO_4$
(5)	$\frac{Ca O}{Mg O}=\frac{5}{1}$	0.5‰ Ca O = 1.465 „ $Ca (NO_3)_2$ 0.1 „ Mg O = 0.298 „ $Mg SO_4$

The pots in each of the four series received 330c.c of the above mineral solution respectively.

Since the evaporation of water from sand is rapid, a special arrangement was provided to secure a constant supply of water consisting in a band of loose cotton freed from the adhering fat, and immersed with one end in water. These bands encircled the pots; thus moisture due to capillary attraction was present always in the pots.

For my experiments the growth of onion plant was observed in these soil and sand mixtures.[1]

[1] Experiments with oats, and bean and bukwheat also had bean commenced but unfortunately insect pests and parasitic fungi caused so much damage that I was compelled to abandon them.

Experiment with the onionplant in sand culture.

10 seeds were sown, on March 13, and the number of shoots reduced to five of nearly equal size on April 1.

The height of the young plants was measured, April 29 with the following results:

	Ratio of Ca O : Mg O.				
	1 : 1	2 : 1	3 : 1	4 : 1	5 : 1
	8.0 cm	10.5	7.7	8.2	8.0
	7.8 „	10.2	7.0	8.2	7.3
	7.4 „	9.8	6.8	7.4	7.0
	6.0 „	8.6	6.1	7.2	6.9
	5.6 „	7.9	6.0	6.9	6.8
average	6.84 „	9.30	6.72	7.52	7.20

A photograph was taken, on May 1, see Plate XI

This result shows the ratio $\frac{Ca O}{Mg O}=2$ the best for onion.

Experiments with Onionplants in Soil from Kawasaki.

Fifteen seeds of onion sown on May 2.

The rate of germination was as follows:

Date	$\frac{Ca O}{Mg O}=\frac{0.75}{1}$	$\left(\frac{Ca O}{Mg O}=\frac{2}{1}\right)$	$\left(\frac{Ca O}{Mg O}=\frac{3}{1}\right)$	$\left(\frac{Ca O}{Mg O}=\frac{4}{1}\right)$
May	3	—	5	5
„ 10	6	6	10	14
„ 12	8	10	13	14
„ 14	12	14	14	14

The number of the young shoots was reduced to 5 on May 15. The number of branches and the height of the young plants were measured on June 30, with the following results:

Ratio $\frac{CaO}{MgO}$		Number of branches	Length, cm.
$\frac{0.75}{1}$	a	4	22.5
	b	5	29.0
	c	4	22.5
	d	4	25.5
	e	4	27.0
	sum	21	average 25.3
$\frac{2}{1}$	a	5	35.0
	b	6	36.0
	c	5	34.5
	d	4	23.0
	e	5	34.0
	sum	25	average 33.3
$\frac{3}{1}$	a	4	31.5
	b	4	31.0
	c	5	25.0
	d	5	24.5
	e	5	29.0
	sum	23	average 28.2
$\frac{4}{1}$	a	4	27.4
	b	5	28.0
	c	4	26.7
	d	5	32.0
	e	3	18.0
	sum	21	average 26.4

These onion plants were harvested on July 12, and yielded the following results:

Ratio $\frac{CaO}{MgO}$		Length of shoots, cm	Number of shoots	Total weight, g.
$\frac{0.75}{1}$	a	29.40	5	2.65
	b	35.70	5	3.40
	c	27.30	4	1.95
	d	30.30	4	2.30
	e	33.75	5	4.10
	average	31.29	sum 23	sum 14.40
$\frac{2}{1}$	a	42.90	5	4.80
	b	44.10	6	7.15
	c	42.60	5	4.51
	d	39.00	5	4.60
	e	41.10	5	4.45
	average	41.54	sum 26	sum 25.51
$\frac{3}{1}$	a	39.00	5	4.05
	b	36.60	5	4.00
	c	30.60	5	2.60
	d	29.70	5	3.15
	e	36.60	5	4.50
	average	36.50	sum 25	sum 18.30
$\frac{4}{1}$	a	36.00	5	3.50
	b	36.60	6	5.15
	c	33.20	5	4.15
	d	37.80	5	4.25
	e	21.90	3	1.50
	average	32.50	sum 24	sum 18.55

Experiments with Onionplant in Soil from Komaba.

Fifteen seeds of onion were sown on May 2.
The rate of germination was as follows:

Date	$\left(\frac{CaO}{MgO}=\frac{1}{1}\right)$	$\left(\frac{CaO}{MgO}=\frac{2}{1}\right)$	$\left(\frac{CaO}{MgO}=\frac{3}{1}\right)$	$\left(\frac{CaO}{MgO}=\frac{4}{1}\right)$
May 8	—	2	2	2
„ 10	1	3	2	5
„ 12	3	5	8	8
„ 14	5	8	8	8

The number of young shoots were reduced to 5 on May 15. The number of branches and the height of the young plants were measured on June 30, with the following results;

Ratio $\frac{CaO}{MgO}$		Number of branches		Length, cm	
$\frac{1.2}{1}$	a	4		28.5	
	b	4		34.0	
	c	4		24.0	
	d	3		19.0	
	e	4		20.5	
	sum	19	average	25.2	
$\frac{2}{1}$	a	5		33.5	
	b	4		34.5	
	c	5		34.8	
	d	5		29.0	
	e	5		36.0	
	sum	24	average	33.5	

Ratio $\frac{CaO}{MgO}$		Number of branches.		Length, cm.	
$\frac{3}{1}$	a	4		36.8	
	b	5		33.5	
	c	3		28.5	
	d	4		30.0	
	e	5		36.5	
	sum	21	average	33.0	
$\frac{4}{1}$	a	4		22.0	
	b	4		31.5	
	c	5		35.0	
	d	3		28.0	
	e	5		28.5	
	sum	21	average	31.0	

On harvesting (July 12) the following result was obtained;

Ratio $\frac{CaO}{MgO}$		Length of shoots, cm.	Number of shoots.		Total weight, g.	
$\frac{1.2}{1}$	a	37.80	4		2.30	
	b	38.40	5		2.30	
	c	30.90	4		1.50	
	d	21.60	4		0.45	
	e	28.50	4		2.00	
	average	31.44	sum	21	sum	8.55
$\frac{2}{1}$	a	40.50	5		5.60	
	b	36.60	4		4.50	
	c	42.00	5		4.70	
	d	36.00	5		3.20	
	e	43.80	5		6.25	
	average	39.78	sum	24	sum	24.25

Ratio $\frac{CaO}{MgO}$		Length of shoots, cm.	Number of shoots.	Total weight, g.
$\frac{3}{1}$	a	41.40	5	5.20
	b	39.90	5	4.80
	c	41.40	4	3.75
	d	37.80	5	3.45
	e	36.00	5	2.75
	average	39.34	sum 24	sum 19.95
$\frac{4}{1}$	a	36.6	5	3.30
	b	36.6	5	4.10
	c	42.0	5	3.95
	d	37.8	4	3.10
	e	37.8	5	3.80
	average	38.01	sum 24	sum 18.25

Hence we find in all these cases that the best ratio of $\frac{CaO}{MgO}$, or the lime factor for the onionplant is = 2.

The chief results with two different soils agree therefore very well with the results in sandculture, where the total content of lime and magnesia was certainly present in an easily available form. It may be safely concluded that the modification I proposed to determine the available amounts of lime and magnesia in soils is in close agreement with the actual results and furnishes therefore a reliable basis for the calculations regarding the liming of soils.

It might be objected that the conditions of the absorptive powers in certain soils might alter the availability of lime and magnesia for the roots. But in my investigation the two soils applied differed very much in character and nevertheless yielded results which agree with each other and with those of the sand culture.

Only soils with a unusually high percentage of clay or humus might yield results differing somewhat from my expectations, but such soils are from the outset not favorable for agriculture.

The experiments of the year 1902 just mentioned were repeated in

1903. The same pots served for this second series, the soil was not renewed, but it was manured again uniformly with 20g KH_2PO_4 and 15g $(NH_4)_2SO_4$ for each 5 kilo. The pots were kept in the green house.

Second experiment with onion plants in the soil from Kawasaki.

15 seeds were sown per pot, on January 7, and the number of the young shoots reduced, on March 2, to six per pot, all being of equal size. The height of the young plants and the number of branches was measured, on May 1, with the following result:

Ratio $\frac{CaO}{MgO}$		Number of branches.	Length cm.
$\frac{0.75}{1}$	a	4	32.0
	b	4	33.0
	c	4	29.5
	d	3	30.5
	e	5	27.0
	f	4	34.0
	sum	24	average 31.0
$\frac{2}{1}$	a	4	33.0
	b	4	32.5
	c	5	46.0
	d	5	32.5
	e	4	31.5
	f	4	34.0
	sum	26	average 34.9
$\frac{3}{1}$	a	4	34.0
	b	4	32.5
	c	4	30.5
	d	4	38.0
	e	5	33.0
	f	4	29.0
	sum	25	average 32.7

Ratio $\frac{Ca\,O}{Mg\,O}$		Number of branches.	Length cm.
$\frac{4}{1}$	a	4	30.0
	b	4	30.5
	c	3	25.5
	d	4	32.5
	e	4	29.0
	f	4	35.5
	sum	23	average 30.5

Up to this time, almost every day 200CC water for irrigation was applied, but the quantity was gradually increased to 300CC.

On harvesting, on June 7, the following result was obtained:

Ratio $\frac{Ca\,O}{Mg\,O}$		Number of branches.	Length cm.	Total fresh weight, grm.
$\frac{0.75}{1}$	a	6	58.0	22.5
	b	6	61.0	17.5
	c	5	57.0	17.0
	d	6	57.0	30.5
	e	7	52.0	14.5
	f	6	62.0	22.0
	sum	36	average 57.8	sum 124.0
$\frac{2}{1}$	a	6	59.0	24.5
	b	8	58.0	31.0
	c	7	74.0	49.5
	d	8	57.0	19.5
	e	7	60.0	28.0
	f	7	61.0	25.5
		43	average 61.5	sum 178.0
$\frac{3}{1}$	a	7	60.0	29.0
	b	6	62.0	24.5
	c	7	63.0	25.0
	d	7	59.0	31.0
	e	6	63.0	25.0
	f	6	45.0	17.5
		39	average 58.6	sum 152.0

Ratio $\frac{Ca\,O}{Mg\,O}$		Number of branches.	Length, cm.	Total fresh weight, grm.
$\frac{4}{1}$	a	5	55.0	25.5
	b	6	57.0	25.0
	c	7	60.0	22.0
	d	6	55.0	22.0
	e	5	52.0	16.0
	f	7	60.0	28.0
	sum	36	average 56.5	sum 138.5

These figures, as well as the photograph taken shortly before harvesting and reproduced on Plate XI show plainly again the superiority of the ratio $\frac{Ca\,O}{Mg\,O}=\frac{2}{1}$ for the onion plants.

Second experiment with onion plants in soil from Komaba.

15 seeds were sown into each pot, on Febr. 10, and the number of the young shoots reduced, on March 20, to six of equal size.

The height of the young plants and the number of branches were measured, on May 12, with the following result:

Ratio $\frac{Ca\,O}{Mg\,O}$		Number of branches.	Length, cm.
$\frac{1}{1}$	a	5	36.0
	b	5	41.0
	c	3	37.0
	d	4	36.0
	e	4	40.0
	f	4	37.0
	sum	25	average 37.8
$\frac{2}{1}$	a	6	47.5
	b	5	40.0
	c	5	45.0
	d	5	45.5
	e	6	43.0
	f	3	38.0
		30	43.1

Ratio $\frac{Ca\,O}{Mg\,O}$		Number of branches.	Length, cm.
$\frac{3}{1}$	a	5	45
	b	3	40
	c	5	38
	d	5	40
	e	4	42
	f	3	38
	sum	25	average 40.6
$\frac{4}{1}$	a	5	40.0
	b	5	41.0
	c	5	39.5
	d	4	37.5
	e	3	42.0
	f	3	40.0
		25	

A photograph was taken shortly before harvesting, on June 15; it is reproduced on Plate XI.

On harvesting, the following result was observed:

Ratio $\frac{Ca\,O}{Mg\,O}$		Number of branches.	Length, cm.	Total weight, grm.
$\frac{1}{1}$	a	5	45	18.5
	b	5	48	18.5
	c	4	47	12.5
	d	5	45	10.5
	e	5	49	15.0
	f	5	47	8.5
	sum	29	average 45.5	sum 83.5
$\frac{2}{1}$	a	7	63	28.5
	b	6	53	19.0
	c	6	58	22.5
	d	6	58	25.5
	e	6	55	22.0
	f	5	56	17.2
	sum	36	average 57.2	sum 134.7

Ratio $\frac{Ca\,O}{Mg\,O}$		Number of branches.	Length cm.	Total weight grm.
$\frac{3}{1}$	a	6	59	19.0
	b	5	53	15.5
	c	6	50	19.0
	d	5	50	18.0
	e	5	50	21.0
	f	5	53	18.0
	sum	32	average 52.5	sum 110.5
$\frac{4}{1}$	a	6	50	19.7
	b	5	52	21.0
	c	6	54	23.0
	d	5	53	17.0
	e	5	55	16.0
	f	5	45	16.0
	sum	32	average 51.5	sum 112.7

Also this result shows that the lime factor for the onion plant is = 2.

Second series of Sandculture.

A large amounts of sea sand (particles smaller than 1 mm. diameter) was soaked in conc. HCl for about a month, and well washed with distilled water until every trace of chlorine reaction had disappeared, then left to dry.

Five pots received each 1.5 litres of this air dry sand, and 80c.c of nutritive solutions which had the following composition:

$$\left.\begin{array}{ll} 0.2\,\% & KH_2PO_4 \\ 0.1\,\,,, & Na_2SO_4 \\ 0.01\,,, & Fe\,SO_4 \end{array}\right\} = a.$$

(1)	$\frac{Ca\,O}{Mg\,O} = \frac{1}{1}$	0.3 % Ca O = 0.879% Ca $(NO_3)_2$ 0.3 ,, Mg O = 1.098 ,, Mg $(NO_3)_2$ + a
(2)	$\frac{Ca\,O}{Mg\,O} = \frac{2}{1}$	0.4 % Ca O = 1.117% Ca $(NO_3)_2$ 0.2 ,, Mg O = 0.732 ,, Mg $(NO_3)_2$ + a

(3)	$\frac{\text{Ca O}}{\text{Mg O}}=\frac{3}{1}$	0.45% Ca O = 1.318% Ca $(NO_3)_2$ 0.15 „ Mg O = 0.549 „ Mg $(NO_3)_2$ +a
(4)	$\frac{\text{Ca O}}{\text{Mg O}}=\frac{4}{1}$	0.48% Ca O = 1.406% Ca $(NO_3)_2$ 0.12 „ Mg O = 0.439 „ Mg $(NO_3)_2$ +a
(5)	$\frac{\text{Ca O}}{\text{Mg O}}=\frac{5}{1}$	0.5 % Ca O = 1.465% Ca $(NO_3)_2$ 0.1 „ Mg O = 0.366 „ Mg $(NO_3)_2$ +a

At the beginning of this experiment, the total concentration of the mineral nutrients for each pot was 2.14—2.38 per mille. The pots were kept in the green house.

20 seeds of onion were sown, per pot, on March 6, and the number o young shoots reduced, on April 7, to 8 of equal size.

The height of the young plants and the number of branches was measured May 1 with the following result:

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$		Number of branches.		Length, cm.
$\frac{1}{1}$	a	4		21.5
	b	5		23.0
	c	5		25.5
	d	5		23.5
	e	4		25.3
	f	3		18.5
	g	4		22.7
	h	4		21.2
	sum	34	average	22.6
$\frac{2}{1}$	a	5		30.1
	b	4		24.0
	c	5		27.8
	d	5		26.2
	e	4		24.9
	f	5		25.0
	g	4		28.9
	h	5		26.2
	sum	37	average	26.6

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$		Number of branches.		Length, cm.
$\frac{3}{1}$	a	4		21.2
	b	4		27.5
	c	4		20.5
	d	5		25.5
	e	5		18.0
	f	5		26.5
	g	4		24.0
	h	5		23.9
	sum	36	average	23.4
$\frac{4}{1}$	a	5		26.0
	b	4		20.2
	c	4		21.5
	d	5		25.5
	e	4		28.3
	f	5		28.5
	g	4		22.3
	h	5		18.6
	sum	36	average	23.9
$\frac{5}{1}$	a	4		18.2
	b	5		21.7
	c	5		26.5
	d	4		26.1
	e	4		22.0
	f	4		17.0
	g	4		18.3
	h	3		18.7
	sum	33	average	21.8

The mineral solutions above mentioned were added in the following proportions:

Date.	March 9	April 20	May 15	June 7
Quantity.	25 c.c	30	40 c.c	40 c.c

In the begining of this culture, every day 25c.c distilled water for irrigation was applied, and the quantity was gradually increased to 70c.c, but sometimes to 100c.c in very warm weather.

The plants were harvested June 27, and the following results observed:

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$		Number of branches.	Length, cm.	Total weight, grm.
$\frac{1}{1}$	a	8	30.1	10.8
	b	6	31.4	8.2
	c	7	25.5	10.7
	d	4	34.0	6.3
	e	6	37.2	10.3
	f	3	22.3	1.9
	g	5	26.1	5.3
	h	5	26.9	11.1
	sum	44	average 29.2	sum 64.6
$\frac{2}{1}$	a	7	30.2	9.2
	b	5	30.0	6.8
	c	7	40.3	9.5
	d	7	33.1	10.0
	e	7	35.2	11.3
	f	5	35.3	9.0
	g	6	37.4	6.5
	h	7	38.0	11.6
	sum	51	average 34.9	sum 73.9
$\frac{3}{1}$	a	7	22.1	6.3
	b	6	30.2	10.5
	c	8	24.7	8.7
	d	7	35.0	10.7
	e	6	30.5	8.8
	f	5	38.1	8.9
	g	4	31.3	6.5
	h	6	28.9	6.4
	sum	49	average 29.7	sum 66.8

Ratio $\frac{\text{Ca O}}{\text{Mg O}}$		Number of branches.	Length, cm.	Total weight, grm.
$\frac{4}{1}$	a	4	30.3	4.7
	b	5	23.5	5.7
	c	7	27.1	7.8
	d	7	30.4	9.0
	e	8	31.0	8.4
	f	8	29.2	7.5
	g	5	29.0	10.2
	h	7	28.0	11.2
	sum	51	average 28.6	sum 64.5
$\frac{5}{1}$	a	7	29.1	9.7
	b	8	29.6	12.2
	c	6	31.3	8.7
	d	4	27.0	5.3
	e	6	23.2	7.2
	f	4	29.5	5.7
	g	6	26.1	7.1
	h	5	30.2	8.0
	sum	46	average 28.2	sum 63.9

Additional Experiments.

Experiment with pea in the soil from Kawasaki.

All principal conditions were the same as in the first onion experiments above described. 15 seeds of pea were sown, on Febr 9 and the number of young plants reduced to five of equal size on March 18. The formation of flowers commenced on May 3 and ended on the 24th of the same month. Up to the flowering period, almost every day 400c.c. of water for irrigation were applied but later on the quantity was increased to 600c.c. When the fruits had reached the ripening stage, on June 7, watering was stopped and

the plants left to dry. The harvest on June 15 yielded the following result:

Ratio $\frac{Ca\,O}{Mg\,O}$	average length.	number of branches.	weight of fresh fruits.	weight of air dry seeds.	weight of air dry straw.	air dry total weight.
$\frac{0.75}{1}$	98 cm	7	18.3 grm	15.7 grm	11.8 grm	29.0 grm
$\frac{2}{1}$	115 „	9	33.0 „	27.5 „	17.0 „	49.3 „
$\frac{3}{1}$	129 „	10	38.3	31.3	21.5	58.0
$\frac{4}{1}$	125 „	9	38.5	31.0	20.4	57.3

Experiment with pea in soil from Komaba.

The number of plants, time of sawing and harvesting was the same as in the case just described.

On harvesting, on June 15, the following results was obtained:

Ratio $\frac{Ca\,O}{Mg\,O}$	average length.	number of branches.	weight of fresh fruits.	weight of air dry seeds.	weight of air dry straw.	air dry total weight.
$\frac{1}{1}$	120 cm	8	35.5 grm	29.7 grm	18.5 grm	52.8 grm
$\frac{2}{1}$	125 „	11	38.0	31.8	23.0	59.4
$\frac{3}{1}$	137 „	11	41.5	33.9	27.4	66.7
$\frac{4}{1}$	123 „	11	36.5	31.2	22.6	58.7

Both results of experiments with different soils show that the ratio $\frac{3}{1}$ is the best for the pea.

Experiment with oats in soil from Kawasaki.

15 seeds were sown December 15, and the number of young shoots reduced to six all of equal size, on March 6; all conditions were essentially the same as in the former cases.

On harvesting (June 29) the following result was obtained;

Ratio $\frac{Ca\,O}{Mg\,O}$	Average height.	Number of stems.	Weight of seeds.	Total weight.
$\frac{1}{2}$ [1]	94	26	41.4	118
$\frac{1}{1}$	105	28	46.1	143
$\frac{2}{1}$	103	28	45.7	141
$\frac{3}{1}$	101	26	42.9	115

Experiment with oats in the soil from Komaba.

These experiments were made at the same time as those with onion. The result was as follows;

Ratio $\frac{Ca\,O}{Mg\,O}$	Average height.	Number of stems.	Weight of seeds.	Total weight.
$\frac{1}{2}$ [2]	112	21	40.1	99.5
$\frac{1}{1}$	117	24	43.3	113
$\frac{2}{1}$	114	25	43.8	117
$\frac{3}{1}$	107	18	37.6	103

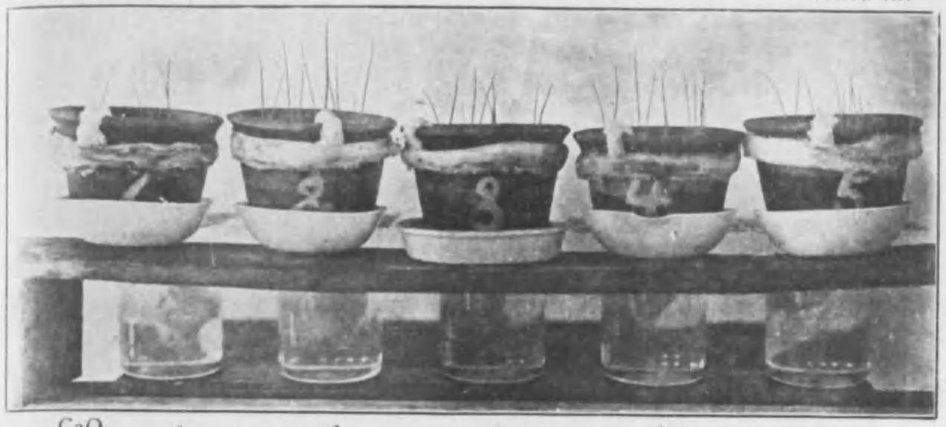
Both results of experiments with oats in different soils show that the ratios $\frac{1}{1}$ and $\frac{2}{1}$ are the best.

[1] In order to procure the ratio $\frac{1}{2}$, this pot received 15,9 gr Mg O = 33.1 gr Mg CO_3.

[2] In order to procure the ratio $\frac{1}{2}$, this pot received 21,7 gr Mg O = 45.2 gr Mg CO_3.

Conclusion.

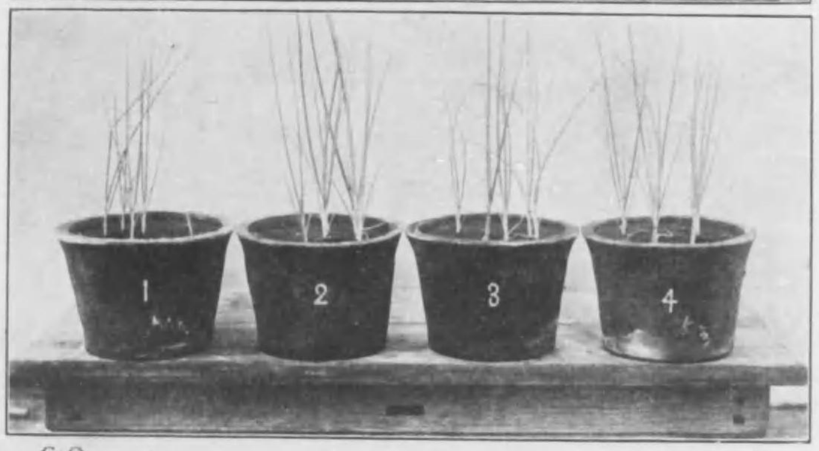
It will be noticed from the second series of experiments with onions, that the results fully confirm the results of the first series that is: Sand-culture as well as the cultures in two soils differing widely in character from each other yielded the best results when the available amounts of lime and magnesia were present in the ratio 2 : 1, in other words the onion has the limefactor 2. Lime and magnesia in the sand culture were added in form of solutions, hence the total amount of these salts were easily available even if precipitated as finely divided phosphates. As to the soil culture the "available amounts" of lime and magnesia were determined according to my modification of the usual method and their ratios changed by adding carbonate of lime in such quantities as to reach the fixed ratios of the sandculture. Since in all my experiments of 1902 and of 1903 the ratio $\frac{CaO}{MgO} = \frac{2}{1}$ proved the most favorable for the onion plant, the determination of the available amounts must have been made by a reliable method. Hence my modification of the usual determination of the available amounts of lime and magnesia may be stated again: I propose to separate all particles <0.25 m.m, to determine the percentage of this fraction, and to extract this fraction for 50 minutes with boiling hydrochloric acid of 10% in the ratio of 25g : 50c.c.



Sand culture. To page 107.

$\frac{CaO}{MgO}$ $\frac{1}{1}$ $\frac{2}{1}$ $\frac{3}{1}$ $\frac{4}{1}$ $\frac{5}{1}$

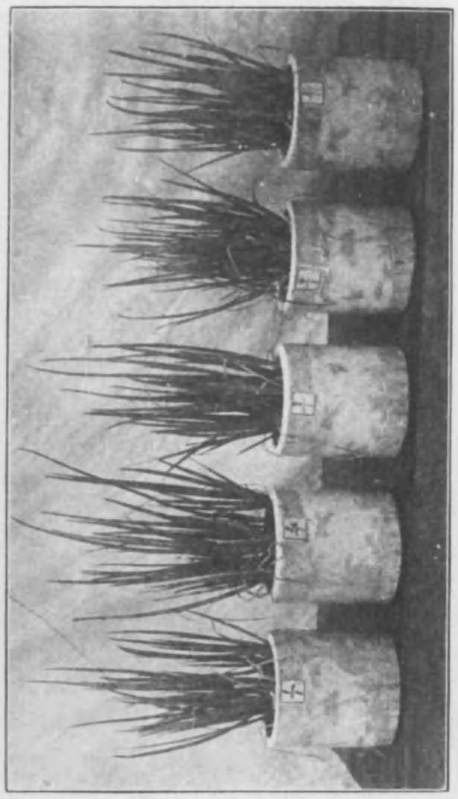
Soil of Kawasaki. To page 109.



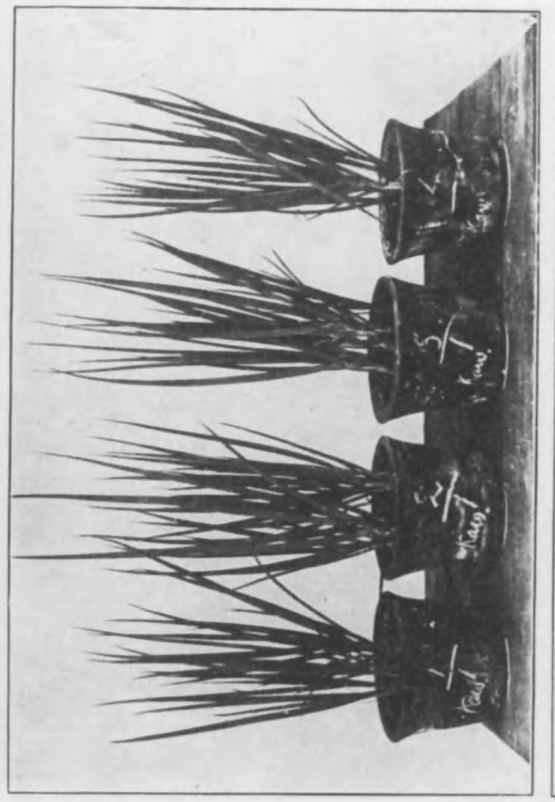
Soil of Komaba. To page 111.

$\frac{CaO}{MgO}$ $\frac{1}{1}$ $\frac{2}{1}$ $\frac{3}{1}$ $\frac{4}{1}$

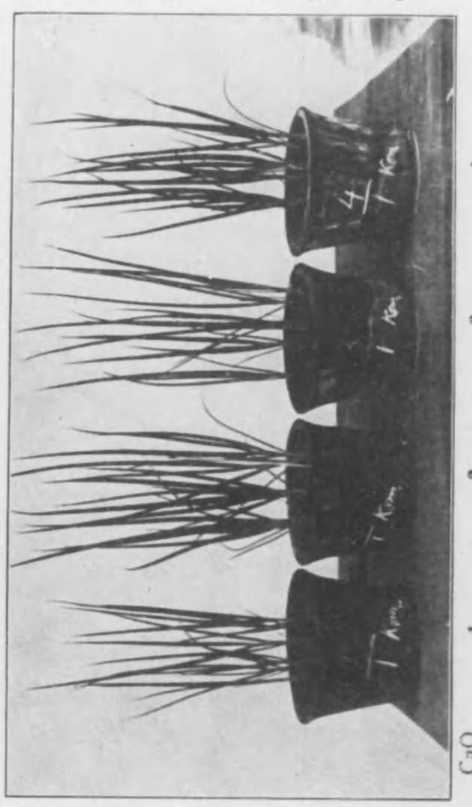
Experiments of 1901.



Sand culture. To page 120.



Soil of Kawasaki. To page 115.



Soil of Komaba. To page 116.

Experiments of 1902.

Ueber den Einfluss des Mangans auf Waldbäume.

VON

Oscar Loew und Seiroku Honda.

Da Manganverbindungen einen günstigen Einfluss auf landwirtschaftliche Gewächse äussern,[1] war es wünschenswerth, auch über den Grad des Einflusses auf Waldbäume einige Anhaltspunkte zu gewinnen. Manganoxyd ist schon häufig in der Asche verschiedener Hölzer, in Blättern und Früchten verschiedener Bäume gefunden worden, die Mengen desselben variirten aber je nach dem Standorte ebenso wie die Mengen des Eisenoxyds beträchtlich,[2] wie die folgenden Daten, welche mit Ausnahme der letzten Zahlen den Aschentabellen *Wolff's* entnommen sind, erkennen lassen:

Object.	Procente in der Asche.		Analytiker.
	Mn_3O_4	Fe_2O_3	
Weidenholz	0.15	0.53	Reichard
Birkenholz I	3.94	3.00	Wittstein
Birkenholz II	4.13	0.59	Berthier
Lindenholz	0.88	0.15	„

[1] Siehe diese Bulletins, Bd. 5.

[2] Nach *Wolff* (Aschenanalysen, 2 Teil, S. 159) nimmt der Mangangehalt der Bäume zu, wenn es an Kalk mangelt, was von *Councler* bestätigt wurde (Zeitschr. f. Forst=und Jagdwesen, Bd. 14, S. 113 [1882] und Bd. 35, S. 391 [1903]. Allerdings beobachtete letzterer auch, dass in den kalkreichsten Organen (Nadeln) der Waldbäume auch der Mangangehalt grösser war als in den anderen Theilen der Bäume.

Object.	Procente in der Asche.		Analytiker.
	Mn_3O_4	Fe_2O_3	
Fichtenrinde	0.65	2.67	Wittstein
Kirschbaumrinde	1.02	0.09	Palm
Ulmenrinde	0.68	0.90	Zeyer
Buchenblätter I	11.25	1.07	Fresenius
Buchenblätter II	1.86	12.00	Wittstein
Weidenblätter	0.29	0.96	Reichard
Birkenblätter	6.73	1.14	Wittstein
Kastanienfrucht	5.48	1.03	Richardson
Buchensamen	3.10	2.66	Souchay
Fichtenpollen	1.12	1.95	Ramann

Man ersieht hieraus, dass der Mangangehalt nicht selten den Eisengehalt weit übertrifft. Dass dieser Mangangehalt irgend einen Einfluss auf die Waldbäume äussern könne, war unbekannt; man hielt ihn für unwesentlich und für zufällig aus dem Boden aufgenommen.

Wir wählten zu unsren Versuchen die für Japan so wichtige *Cryptomeria japonica*. Am 29 April 1902 pflanzten wir auf Beeten von zwei Quadratmeter Grösse die jungen nahezu gleichgrossen Bäume ein. Zu dem Versuch dienten sechs solche Beete, welche von einander durch eine etwa 1 Meter breite mit niederen Brettern abgegränzte Fläche getrennt waren. Der Boden war ein humoser Lehmboden von nur mässiger natürlicher Fruchtbarkeit. Jedes Beet erhielt anfangs neun Pflanzen, die aber später auf acht reducirt wurden, da einige eingiengen. Auf Beet No. 6 waren nach einiger Zeit nur sieben Pflanzen erhalten. Die Beete wurden genau wie alle jungen Pflanzungen der Förstereien behandelt und im Winter gegen Frost geschützt. Die Loesungen wurden nicht gleichmässig über die Beete verbreitet, sondern in eine kleine Vertiefung um jeden Stamm die berechnete Menge gegossen. Die Loesungen wurden zehnprocentig vorrätig gehalten und die jedesmal nötige Menge auf das hundertfache verdünnt.

Beet No. 1 erhielt von 1 Mai bis 1 November 1902 allmonatlich 0.5g Mangansulfat, nur bei der ersten Begiessung die doppelte Menge; im Jahre 1903 aber vom 1 Mai bis 1 Nov. (inclusive) allmonatlich 1g. Es erhielt also jede der 8 Manganpflanzen im Ganzen 1.5 gramm jenes Salzes.

Beet No. 2 erhielt Eisensulfat (Eisenvitriol) in gleicher Weise und Menge wie Beet No. 1 das Mangansulfat. Beide Sulfate waren die krystallisirten, nicht chemisch reinen, Producte des Handels.

Beet No. 3 erhielt lediglich die jenen Loesungen entsprechende Menge Wasser, und diente wie Beet No. 5 und No. 6 als Controlbeet.

Beet No. 4 erhielt Kochsalz, No. 5 Natriumnitrat, No. 6 Calciumnitrat in denselben Mengen wie Beet No. 1 das Mangansulfat. Diese Nitrate wurden angewandt, um zu beobachten, ob eine teilweise Düngung eine ähnliche Wirkung äussern könnte, wie Mangansulfat. Das Chlornatrium auf Beet No. 4 sollte Aufschluss geben, ob die Holzbildung befördert wird; denn in der Landwirtschaft ist bei einem gewissen Chlornatriumgehalt des Bodens ein schnellerer Verbrauch von Stärkemehl und Zucker zu Gunsten der Ausbildung der Holzfasern beobachtet worden, oder es wird wenigstens ein derartiger Einfluss des Kochsalzes für wahrscheinlich gehalten.

Vier Monate nach der Behandlung war noch kein deutlicher Unterschied im Höhenwachstum wahrzunehmen, erst im fünften Monat war ein Voraneilen der Manganpflanzen deutlich zu erkennen. Dieses Voraneilen nahm aber im folgenden Frühjahr ein rascheres Tempo an, bis im Herbst des zweiten Jahres ein höchst auffallender Höhenunterschied erreicht wurde, wie aus folgender Tabelle ersichtlich wird. Die Zahlen geben die Höhen in Centimetern an.

No. der Pflanze	Mangansulfat $MnSO_4$ + 4 aq.		Ferrosulfat $FeSO_4$ + 7 aq.		Control		Chlornatrium		Natriumnitrat		Calciumnitrat	
	Mai 1/02	Nov. 10/02	Mai 1/02	Nov. 10/02	Mai 1/02	Nov. 10/02	Mai 1/02	Nov. 10/02	Mai 1/02	Nov. 10/02	Mai 1/02	Nov. 10/02
1	18.0	49.3	19.7	29.5	18.7	33.5	19.5	31.0	18.7	31.0	22.7	36.7
		135		115		130		90		115		110
2	19.5	47.5	22.0	34.0	18.3	35.0	20.7	36.0	19.9	32.0	20.5	24.4
		168		85		89		97		166		54
3	19.7	34.0	19.0	27.3	10.2	19.0	19.6	29.0	18.6	28.5	18.7	35.0
		133		105		45		95		62		65
4	17.0	44.0	21.3	31.2	19.0	27.0	17.8	23.0	19.0	41.5	23.0	32.7
		145		95		110		60		143		111
5	19.5	31.0	17.5	31.5	16.5	34.8	18.7	24.5	20.5	33.0	20.6	33.0
		100		104		105		81		96		110
6	21.5	32.8	20.2	31.5	20.3	30.0	18.5	27.0	18.7	35.0	20.0	32.7
		114		98		99		95		121		103
7	19.5	35.5	18.0	27.3	17.7	25.5	16.3	23.0	18.0	30.5	16.1	20.0
		137		108		90		70		97		71
8	20.3	24.5	18.5	37.5	16.4	21.0	13.7	18.0	17.9	25.0	—	—
		89		138		93		58		77		—

Hieraus beerchnet sich für die Zeit vom 1 Mai 1902 bis 10 Nov. 1903 das durchschnittliche Zuwachsprocent bei Behandlung mit:

Mangansulfat zu	558.7
Ferrosulfat	445.5
Chlornatrium	345.5
Natriumnitrat	426.7
Calciumnitrat	340.6
und für die Controlpflanzen ...	448.2

Man erkennt hieraus, dass *Mangansulfat das Höhenwachstum stark förderte*, dass das Ferrosulfat diese fördernde Wirkung nicht hatte und Chlornatrium sowohl als Calciumnitrat hemmend gewirkt haben.[1] Bemerkenswert ist noch, dass die Düngung mit Chilesalpeter das Höhenwachstum nicht förderte. Indessen der *Eisenvitriol* sowohl als der *Chilesalpeter* haben das *Wachstum der Zweige begünstigt* wie aus dem Vergleich der Totalgewichte erhellt.

Am 17 November wurden die Bäume am Grunde abgesägt und frisch gewogen mit folgendem Resultat bei:

Manganosulfat	5871 gramm.	
Ferrosulfat	3395 „	8 Pflanzen.
Chlornatrium	1390 „	
Natriumnitrat	3355 „	
Calciumnitrat	2497 „	(7 Pflanzen)
Control	2535 „	(8 Pflanzen)

Es ergibt sich daher als Durchschnittsgewicht für eine Pflanze:

bei Manganosulfat	733.8 g
„ Ferrosulfat	424.5 „
„ Chlornatrium	173.8
„ Natriumnitrat	419.4
„ Calciumnitrat	356.7
„ Control	316.9

[1] Auch *Loughridge* (Calif. Stat. Bul. 133) und *Kostewitsch* (Journ. f. Exper. Landw 1903, p. 44) beobachteten einen sehr schädlichen Einfluss von Kochsalz auf Bäume. Natriumsulfat ist nach diesen Autoren bedeutend weniger schädlich.

Die Durchschnittshöhe bei der Pflanzung der Bäumchen war auf dem Manganbeet = 19.4, auf dem Controlbeet aber = 17.1 cm.

Berechnet man nun das Erntegewicht bei den Manganpflanzen auf gleiche Anfangshöhe wie bei den Controlpflanzen um, so hat man für eine Manganpflanze = 646.7g. für die Controlpflanzen gleicher Anfangshöhe = 316.9g.

Die Manganpflanzen hatten also für dieselbe Anfangshöhe nach 1½ *Jahren die Controlpflanzen um das doppelte* (2.03 fach) *an Massenzunahme übertroffen*, wie auch wohl aus der in Tafel XII reproducirten Photographie abgeschätzt werden könnte.[1]

[1] Von einigem Interesse ist noch der Unterschied in der Wirkung von Natriumnitrat und Calciumnitrat, weil solche Unterschiede zu Gunsten des Natriumnitrats auch in der Landwirtschaft beobachtet sind. Es wirkt eben auch das Natron in der Form des Nitrats stimulirend, während Chlornatrium in Folge des Chlorgehaltes wieder hemmend wirkt, wenigstens in grösseren Dosen. In relativ geringerer Menge kann aber auch dieses eine mässig stimulirende Wirkung auf Feldgewächse ausüben. Fichten reagiren nicht so energisch auf Mangan als Cryptomerien. Versuche in grösserem Massstabe wird der eine von uns (Honda) weiter führen.

BULL. AGRIC. COLL. VOL. VI. *PLATE XIII.*

Control plants. Manganese plants.

Plate showing the stimulating effect of manganous sulphate upon the growth of Cryptomeria japonica. To page 130.

On the Practical Application of Manganous Chlorid in Rice-culture.

BY

K. Asō.

In the last volume of this Bulletin, several communications regarding the stimulating action of manganese upon plant-growth were published. Loew and Sawa[1] observed this action with plants in water and soil culture and also the author[2] with various plants in waterculture. Nagaoka[3] further carried out a field experiment with rice in wooden frames and obtained an increase of one third of the harvest in grains by the application of manganous sulphate at the rate of 35 kilo $Mn_3 O_4$ per hectar. In all these experiments, mangano sulphate was used. But, since manganous chlorid is a cheap by-produrt in the bleaching powder factories, it seemed to me of some practical importance to make also an experiment with this salt.

My experiment was carried out in the paddy field with rice in quite the same manner as the practical farmer does. Two square-shaped plots, each of 30 sq. metre, were selected in a field which had not been manured for several years. Each plot received 27 kilo barnyard manure, 15.5 kilo rotten human excrement, 230 grams double superphosphate and aftwards 570 grams wood ash. Besides, one plot received 200 grams crystallized manganous chlorid[4] (corresponding to 25 kilo $Mn_3 O_4$ per ha.), while the other served as control.

On July 3, the young rice plants[5] from the seed-bed were transplanted,

[1] Bul. College. Agric. Tokyō. Vol. V. No. 2.
[2] ibid. No. 2.
[3] ibid. No. 4.
[4] This was applied separately after manuring.
[5] The variety was the Satsuma.

each plot receiving 305 bundles of twelve equally developed individuals. The irrigation and the drainage were made in each plot separately in the same manner as practically carried on and care was taken to avoid the passing of drainage water from one plot to the other.

Towards the end of July a difference in regard to the development was quite marked and became gradually more noticeable. On September 3, all plants in the manganese plot flowered and four days later in the control plot. The weather conditions were very favorable for rice culture throughout the whole summer, and all possible attention was paid to avoid damages by insect pests. On November 6, the plants were harvested.

The harvest was weighed in the air dry state:

	Manganous chlorid.	Control.
Total harvest	23.74 k.	16.73 k.
Straw	12.10 k.	8.19 k.
Total grains	11.49 k.	8.46 k.
Full grains	11.23 k.	8.23 k.
Empty grains	0.26 k.	0.23 k.
Husked full grains	8.66 k.	6.65 k.
Weight of 1 Litre of unhusked full grains.	616 g.	619 g.

Now, if the yield of the control plot is taken as unit, the following figures are obtained:

	Manganous chlorid plot.
Total yield	1.42
Straw	1.48
Full grains (unhusked)	1.36
Full grains (husked)	1.30

These figures show an increase of one third of the grains by the application of 25 kilo. Mn_2O_3 per hectar in the form of manganous chlorid,[1] which is in full coincidence with the result of Prof. Nagaoka who had applied the same amount of Mn_2O_3 in the form of the sulphate. Since the area of one plot corresponded to $\frac{1}{2\frac{1}{2}0}$ hectar and the quantity of manganous chlorid applied was 200 grams, 66 kilo of this salt would be required per hectar. The cost would be only 4.4 yen,[2] while the value of the increased harvest is 137.33 yen.[3]

There experiments will be continued for a series of years on the same plots.

[1] As to pot cultures manganous sulphate would be preferable to the chlorid, since chlorids often exert an injurious influence on the yield. In field culture—especially with paddy field—this depressing factor is removed by rains and irrigation water. The manganous chlorid of course changes in the soil into other manganese compounds.

[2] The price of 100 pounds of the crystallized salt $MnCl_2+4aq.$ is three yen (about six Mark).

[3] Compare Bul. College. Agric. Tokyō. Vol. V. No. 4. p. 472.

On the Stimulating Action of Manganese upon Rice, II.

BY

M. Nagaoka.

In a former Bulletin (vol. V, No. 4) I had shown that manganese compounds can increase the yield of paddy rice. The experiment was repeated under essentially the same conditions in the same frames as before, the only difference being that no fresh doses of manganese sulphate were applied since this time it was the chief object to *observe any after effects of the first doses* given the previous year. The crop was harvested on Nov. 11 and weighed two months later after being well air dry. The results are seen from the following table:

No. of Frames.	Mn_2O_3 per ha kg.	Full grains gr.	Empty grains gr.	Straw gr.	Average. Full grains.	Average. Empty grains.	Average. Straw.	Total.
1	No manure	136.5	2.1	217.9				
13	and no	196.2	4.0	288.1	186.7	3.4	272.0	462.1
25	Mn_2O_3	227.5	4.0	309.9				
2	No Mn_2O_3	182.0	3.1	271.9				
14		204.7	3.3	295.3	207.6	3.5	295.7	506.8
26		236.0	4.2	319.9				
3		212.5	3.7	293.1				
15	10	179.9	3.7	277.4	203.7	3.9	289.8	497.4
27		217.0	4.4	298.9				
4		221.7	3.6	305.4				
16	15	213.0	4.0	289.9	224.9	4.0	304.6	533.5
28		240.0	4.3	318.4				

No. of Frames.	Mn_2O_3 per ha kg.	Full grains gr.	Empty grains gr.	Straw gr.	Average. Full grains.	Empty grains.	Straw.	Total.
5		238.0	4.1	323.4				
17	20	248.0	4.8	219.4	230.3	4.4	316.4	551.1
27		205.0	4.3	306.4				
6		244.0	5.1	324.8				
18	25	238.0	4.8	315.6	235.5	4.9	308.8	549.2
30		224.5	4.7	285.9				
7		247.4	5.2	329.9				
19	30	251.7	4.9	324.2	242.7	5.0	322.2	569.7
31		229.0	4.9	312.4				
8		196.5	4.6	295.9				
20	35	270.0	4.1	339.7	231.2	4.6	313.5	549.3
32		227.0	5.2	304.9				
9	40	202.9	5.1	298.7	215.0	4.6	306.6	526.2
21		227.0	4.0	314.4				

The harvest in full grains was therefore greatest in the frame that had received manganous sulphate at the rate of 30 kilo Mn_2O_3 per ha; very near to this comes the frame with the ratio of 25 kilo Mn_2O_3 per ha, which in the first year yielded the greatest weight of full grains. The increase over the manured frame without manganese was 16.9% while the maximum increase in the first year was 37%.

On the Influence of Manganese Salts on Flax.

BY

Y. Fukutome.

In former experiments, described in the Bulletins of the College of Agriculture, was shown that manganese salts can exert a stimulant action on various plants serving as food. It seemed interesting to make further observations also on plants that are cultivated for their fibres.

I selected for this purpose flax and compared here the action of manganous chlorid with that of ferrous sulphate and Cobalt nitrate. The soil came from the experiment grounds of our College of Agriculture. Each pot containing eight kilo soil was manured with 16 g. superphosphate, 10 g. potassium sulphate, 8 g. each of ammonium sulphate, and sodium nitrate.

40 seeds were sown in each pot on September 21 and the shoots singled out in October to 15 of equal height (=5 cm.).

Pot I. had received 0.4g crystallized manganous chloride ($Mn\,Cl_2, 4H_2O$)
Pot II. „ 0.4g ferrous sulphate ($Fe\,SO_4, 7H_2O$)
Pot III. „ 0.4g cobalt nitrate ($Co(NO_3)_2, 6H_2O$)
Pot IV. „ 0.02g „ „ „
Pot V. „ 0.4g ($Mn\,Cl_2, 4H_2O$) + 0.4g ($Fe\,SO_4, 7H_2O$)
Pot VI served as Control.

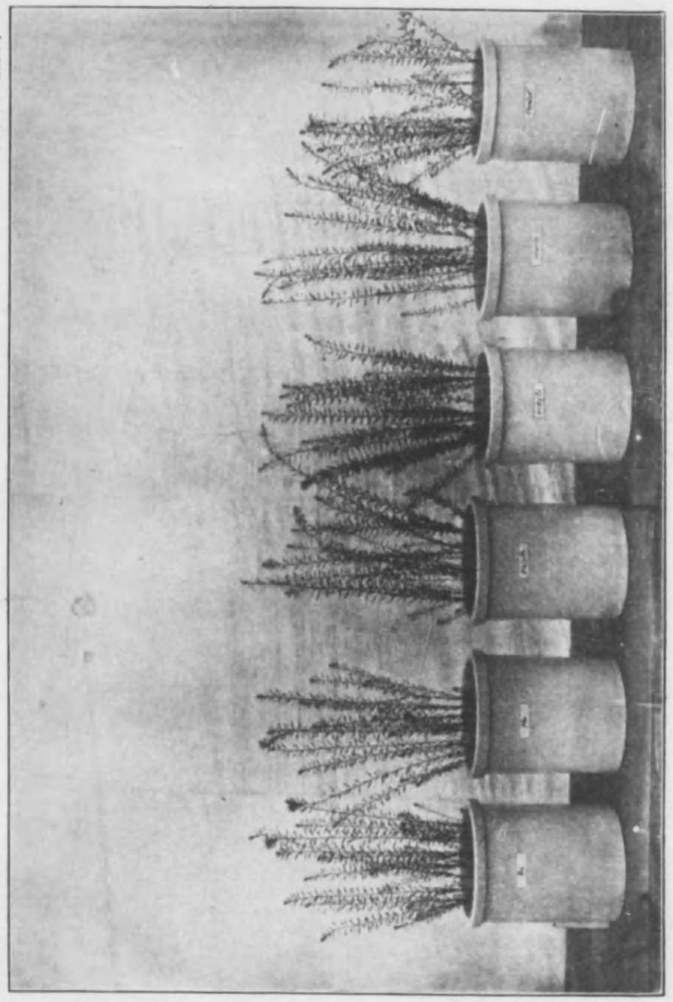
On Nov. 30 the plants were measured and a photograph taken, reproduced on Plate XIII. It shows that a stimulating action had taken place. On Dec. 21 the stem and branches were again measured, whereupon the plants were cut and left to become air dry. At that time only two flowers opened, one had in pot IV and one in pot V.

The results were as follows:

	Nov. 30	Dec. 21				
	Height, cm. average	Height, cm. average	Number of Buds	Number of branches	Average length of branches	Weight, g. (airdry)
I. Mn.....	46.9	65.7	2	19	26.7	10.7
II. Fe.....	47.4	67.8	5	17	24.5	11.6
III. Co (0.4 g.)...	48.6	68.5	3	19	27.4	11.0
IV. Co (0.02 g.)..	48.9	69.6	5	19	33.8	12.8
V. Mn+Fe ...	51.7	71.9	4	19	32.9	12.9
VI. Control.....	43.4	60.1	0	19	25.	10.5

This result shows that the joint application of iron and manganese had a marked effect on the growth, while each alone but little in the dose of 0.4 g per 8 kilo soil. Also cobalt nitrate in the small dose of 0.02 g per pot exerted a stimulating effect.

PLATE XIV.



BUL. AGRIC. COLL. TOK. VI.

Flax under the influence of ferrous sulphate, manganous chlorid and cobalt nitrate.

To page 138.

Can Potassium Bromid Exert any Stimulating Action on Plants?

BY

K. Asō.

Potassium bromid is only a very weak poison for plants, certainly very much weaker than potassium iodid.[1] The question whether very small quantities of that salt can exert any stimulating action was thus far only tested by *Völcker*, who found that barley soaked for a short time in a potassium bromid solution of 1%, yielded afterwards an increased harvest.[2] It was desirable to collect some further information in this regard.

Four pots containing 2.5 k. air dry soil were manured as follows:

Sodium nitrate	3 g.
Ammonium sulfate	1 g.
Monopotassium phosphate	3 g.
Potassium carbonate	3 g.

One pot received 10 mg. potassium bromid, another 100 mg. and the third 500 mg. potassium bromid for each kilo soil, while one pot served as control. On April 23, ten seeds of *Phaseolus* (Dwarf variety) were sown and on May 25, the plants were reduced to two of equal height in each pot.

The plants measured on July 2:

Control plants	Potassium bromid plants		
	10 mg per kilo.	100 mg per kilo.	500 mg per kilo.
cm.	cm.	cm.	cm.
22	22	22	22
21	24	24	19

[1] Cf. O. Loew, Ein natürliches System der Giftwirkungen, p. 108.

[2] Journ. Roy. Agr. Soc. Engl. (III) 11, p. 566.

On July 24, the plants were harvested.

	Control	Potassium bromid		
		10 mg per kilo.	100 mg per kilo.	500 mg per kilo.
Number of fruits (ripe).....	4	7	6	6
,, (unripe).....	2	0	1	2
Weight of pods (air dried)	5.9 g.	11.0 g.	8.8 g.	7.4 g.
Number of ripened seeds.....	15	26	21	21
Weight of ripened seeds (air dried)	4.4 g.	8.5 g.	6.5 g.	5.7 g.

These figures leave no doubt that 10 milligram potasium bromid per kilo soil had exerted a stimulating action and further that this beneficial action decreased with the increase of that salt. But even 500 mg. potassium bromid per kilo soil had still a slight stimulating action.[1]

Another experiment was made with upland rice. The manuring and the quantity of potassium bromid were quite the same as in the case with *Phaseolus*. On April 23, ten seeds of upland rice were sown into each pot and the young plants reduced to seven of equal size on June 4.

Later on two more were removed from each pot on account of damage by fungi. The height on July 24 was:

Control	Potassium bromid		
	10 mg per kilo.	100 mg per kilo.	500 mg per kilo.
cm.	cm.	cm.	cm.
60	80	69	68
68	82	74	68
80	74	69	72
67	71	60	64
67	78	70	58
—	—	—	—
Average length. 66	77	68	68

[1] The seeds of these plants yielded again normal plants.

On October 5, the plants were harvested.

	Control	Potassium bromid		
		10 mg per kilo.	100 mg per kilo.	500 mg per kilo
Average length	81.4 cm.	87.6 cm.	83.6 cm.	75.0 cm.
Weight of grains (air dry).	10.5 g.	11.2 g.	8.7 g.	8.0 g.
Weight of straw (air dry).	18.5 g.	22.2 g.	18.2 g.	14.5 g.

This result shows, like in the former case, that 10 milligram potassium bromid per kilo soil exerts a stimulating action but this action diminishes with the increase of the amount; 100 milligram potassium bromid per kilo soil depressed the weight of grains and 500 milligram potassium bromid injured the normal growth of the plants.

A further experiment was made with fungi. The culture solutions contained:

0.5 % pepton
1.0 % glycerol
0.2 % monopotassium phosphate
0.02% magnesium sulphate.

Potassium bromid was added at the rate of 0.1%, 0.01% and 0.001%. These solutions were infected with a trace of spores of *Aspergillus Oryzae* and kept in diffused day-light at the ordinary temperature.

For control the solutions received equal amounts of potassium chlorid. After four days, the fungus mass was collected on weighed filters and dried. The result was as follows:

		Weight of the fungus mass.
0.1% KCl	a	0.013 g.
	b	0.015 ,,
0.01% ,,	a	0.012 ,,
	b	0.015 ,,

Weight of the fungus mass.

0.001% KCl	a	0.013 g.
	b	0.013 „
0.1% K Br	a	0.010 g.
	b	0.013 „
0.01% „	a	0.022 „
	b	——
0.001% „	a	0.012 „
	b	0.010 „
Control (no KCl and no K Br)		0.018 „

These figures show quite undecisive differences.

Summary.

Potassium bromid at the rate of 10 milligrams per kilo soil exerts a stimulating action on bean and rice. This effect diminishes with the increase of the amount of that salt. With fungi (Aspergillus Oryzae) no stimulating effect of potassium bromid was observed at doses of 0.001—0.1%.

Can Thorium and Cerium Salts Exert any Stimulating Action on Phaenogamous Plants?

BY

K. Asō.

Thorium became recently one of the most interesting elements on accouut of its compounds showing radioactivity.[1] It seemed to me therefore of some interest to observe its action on living plants. In a 1% solution of thorium nitrate ($Th(NO_3)_4$) young barley plants 12—15 cm. high were killed after about one day. This can not surprise us, however, since thorium nitrate is of strong acid reaction which in itself would be sufficient to kill the plants within that time. In a 1 per mille solution of that salt no injurious action whatever was observed on such plants even after eight days. It can therefore be inferred that thorium nitrate has no poisonous properties, which confirms the observation of Bokorny made about ten years ago.

Observations on the action of thorium compounds on plants in full nourishing solutions are hardly possible, since the phosphates would precipitate the thorium as phosphate almost completely, which phosphate settling as a fine powder, remains out of reach by the roots. Hence some experiments with plants in soil culture were instituted.

Two pots containing eight kilo soil received each the following manure:

KCl	5 g.
K_2CO_3	7 g.
$NaNO_3$	6 g.
$(NH_4)_2SO_4$	6 g.
Common superphosphate	18 g.

[1] *Rutherford* and *Soddy* (Chem. News, Vol. 86, No. 2236) assert that radio-activity is a manifestation of subatomic chemical change.

One pot received thorium nitrate 10 mg. and the other 100 mg. for each kilo of soil. The third pot served as control. Twenty seeds of buckwheat were sown March 12. On March 26, the young shoots were thinned out to five of equal size in each pot. On April 30, the plants were measured with the following result:

	Thorium nitrate 10 mg per kilo.	Thorium nitrate 100 mg per kilo.	Control.
	cm.	cm.	cm.
	52	45	50
	55	49	52
	55	52	57
	69	57	61
	70	69	62
Average.....	56.2	54.4	56.4

This shows that there was no stimulating action exerted in regard to the development in height. On July 9, the plants were cut and the straw and grains weighed in the air dry condition with the following result:

	Thorium nitrate 10 mg per kilo.	Thorium nitrate 100 mg per kilo.	Control
	g.	g.	g.
Full grains	17.8	16.0	28.3
Unripe grains ...	4.7	4.5	4.0
Straw	18.7	13.5	20.3

It will be seen that thorium nitrate exerted a depressing influence on buck wheat in a dose of 100 mg. per kilo soil and that even if the quantity is reduced to 10 mg. per kilo soil no stimulating action on buck wheat, in contrary a considerable depression of harvest is obtained.

In order to observe whether plants of other families behave alike, a second experiment was made with a grass, *Panicum frumentaceum.*

In manuring the following doses were applied for each pot of eight kilo soil.

KCl ..... 5 g.
K_2SO_4 ..... 7 g.
$Na\ NO_3$ ..... 6 g.
$(NH_4)_2\ SO_4$ ..... 6 g.
$Na_2\ HPO_4$ ..... 10 g.

The pots and soil used this time were the same as before and therefore no new doses of thorium nitrate were applied this time. On July 24, the seeds were sown and afterwards the plants were reduced to nine in each pot. During the development of these plants, some action was observed in the case of 100 milligram thorium nitrate per kilo soil. Even in the pot which received 0.08 grams thorium nitrate (10 milligram per kilo soil), the plants developed better than in the control case; unfortunately, however, some plants in this pot were damaged by certain insects. On October 5, the plants were harvested with the following results:

	Control	100 mg Thorium nitrate per kilo soil
Average length...	100 cm.	101.4 cm.
Weight of grains (air-dried) ..	10.2 g.	12.0 g.
Weight of straw (air-dried) ...	14.7 g.	23.7 g.

These differences are not very decisive but a slight stimulating action of thorium on Panicum is probable, while with buckwheat no stimulating effect was noticeable.

Experiment with cerium. Three pots, each holding 2.5 kilo air dry soil were manured as follows:

$Na\ NO_3$ ..... 3 g.
$(NH_4)_2\ SO_4$ ..... 1 g.
$KH_2\ PO_4$ ..... 3 g.
$K_2\ CO_3$ ..... 3 g.

Pot (A) received 10 milligr. ceric sulphate per kilo soil; Pot (B) 100 mg, while Pot (C) served as control. On May 4th, 16 seeds of upland rice steeped previously in water for two days were sown and on July 24, the number of plants was reduced to four of equal size in each pot. Till the beginning

of July, the development of plants in B was far behind that of the control plants and even those in A did not surpass the control plants in hight. Here some injurious action was evident. But after the middle of July, the plants in A exceeded the others in height.

	A Ceric sulphate 10 mg per k.	B Ceric sulphate 50 mg per k.	C Control
	cm.	cm.	cm.
	8.7	8.9	8.0
	8.2	7.3	7.7
	7.4	7.2	7.3
	8.0	6.7	8.2
Average	8.1	7.5	7.8

The fact that the action of the ceric sulphate was now more favorable than in the beginning would find a simple explanation, if the poisonous ceric sulphate which has strong oxidizing powers was turned gradually by the organic matter in the soil into cerous sulphate, which has not such an oxidizing power, and conseqnently would be less poisonous. On October 5, the plants were harvested with the following results:

	A Ceric sulphate 10 mg per kilo soil	B Ceric sulphate 50 mg per kilo soil	C Control
Average length	88.5 cm.	95.5 cm.	89.7 cm.
Weight of grains (air dried).	11.2 g.	12.5 g.	10.5 g.
Weight of straw (air dried).	18.0 g.	18.0 g.	18.2 g.

These differences are but small and leave it rather doubtful whether cerium has any stimulating action.

Can Salts of Zinc, Cobalt and Nickel in High Dilution Exert a Stimulant Action on Agricultural Plants?

BY

M. Nakamura.

Since manganese can promote the growth of plants, I believed it to be of some value to observe also the actions of the related elements zinc, cobalt, and nickel in high dilution. The salts of these elements are known to act poisonously in moderate concentration,[1] but whether these may act as stimulants on agricultural plants when very highly diluted has not yet been the object of observation, except quite recently in the laboratory of Prof. Miyoshi.

The results would be of some interest, especially in regard to zinc, since *zinc pots are frequently made use of in agricultural experiments.*

Zinc becomes easily oxidized in presence of moisture and air and the roots of plants growing in zinc pots may come in contact with the oxydized surfaces, and absorb some zinc oxid.

As *Raulin*[2] and others have shown that fungus growth may be enhanced by traces of zinc salts, erroneous conclusions may be arrived at if phænogams would behave in like manner. In regard to algae *One*

[1] *F. Nobbe, P. Baessler* and *H. Will* in Landw. Vers. Stat Bd. XXX and further *A. Baumann* I bid, Bd. XXXI. This author experimented with water and soil cultures and observed with various plants a different degree of resistance-power, further a considerable difference in the absorptive powers of various soils for zinc. Humus in the soil dimnishes the poisonous effects of zinc salts, since they are transformed by it into insoluble compounds.—*Koenig* inferred that waters containing $ZnSO_4$ should in no case serve for irrigation.

[2] *O. Raulin* applied a solution of 70g. sugar and 4g. tartaric acid in 1500g. water in presence of the necessary mineral nutrients. On addition of $\frac{1}{3000}$ zinc sulphate the fungus ogrwth was found 3-4 times that in the control case.

has made some interesting observations.[1] He writes: „Bei unseren Versuchen mit Algen wirkte Zinkvitriol nächst Eisenvitriol sehr günstig auf des Wachstum ein schon schon bei Zusatz von einer minimalen Quantität, wie 0,00006% bis 0,0003%. Stieg die Concentration auf 0,0016%, so litten die Algen nicht unerheblich, ohne dass jedoch das Wachstum ganz unterdrückt worden wäre. Unsere Versuche mit Pilzen stimmen mit denjenigen von *Richards* überein."

„Meine Versuche mit Eisenvitriol zeigen, dass Algen dasselbe in höherer Concentration als andere Schwermetallsalze ertragen. So lag bei Hormidium das Optimum etwa bei 0,0005%, und sogar bei einer höheren Concentration wie 0,0126% war der Ertrag noch etwas grösser als bei den Controlpflanzen. Die optimale Dosis von Nickelvitriol lag etwa zwischen 0,00006 und 0,00012%, während von 0,0028% an eine schädigende Wirkung auf Algen eintrat."[2]

„Bei Algen scheint auch Cobaltsulfat einen begünstigenden Einfluss auszuüben, Optmum bei etwa 0,00012%."

For my own experiments I selected an *Allium*, a small variety of *Brassica chinensis*, further barley and pea. Fach pot contained 2300g. air dry soil and was manured with 3g. sodium nitrate, 3g. potassium carbonate and 4g. double superphosphate.

Pot No. I received—0,01g. Zn SO_4 (0,01782g. Zn SO_4 + 7aq.)
Pot No. II „ —0,01g. Ni SO_4 (0,01813g. Ni SO_4 + 7aq.)
Pot No. III „ —0,01g. $Co(NO_3)_2$(0,01593g. $Co(NO_3)_2$ + 6aq.)
Pot No. IV served as control.

These mineral salts were incorporated in form of a very diluted solution in order to insure a complete distribution through the soil, which was well stirred after the addition. Pot No. 4 received at the same time as much water, as the others the solutions.

[1] Journal of the College of Science, vol 13. Über die Wachstumsbeschleunigung einiger Algen und Pilze durch chemische Reize.

[2] A stimulating effect of zinc sulfate on phaenogams was recently observed by *Kanda* in the Botanical Institute of this University.

Experiment with Allium.

Fifteen seeds were sown September 25 in each pot, and after the young shoots had reached 3-5cm they were reduced to 5 per pot, all of nearly equal height. Towards middle of December it became evident that the leaves of the zinc, nickel and cobalt plants were larger than those of the control plants. Since the growth became gradually very slow and the leaves assumed a yellowish hue, some ammonium sulphate was applied 0,5g. for each pot, February 1. which showed soon a very favorable effect. On February 27 the following observations were made:

	Average length of leaves (cm.)	Number of branches.
Zn—plants	32,6	20
Ni— „	34,0	21
Co— „	33,6	19
Control	31,2	19

The plants were harvested May 1 with the following results:

	Total weight.	Number of branches.	Number of flowers.	Average length.
Zn—plants	155,8 g.	25	5	54 cm
Ni— „	163,5 „	23	5	54,4 „
Co— „	129, „	23	4	52,8 „
Control	123, „	23	2	50,6 „

The result shows indeed some stimulating action of small doses of nickel, cobalt and zink.

Experiment with Brassica Chinensis.

Fifteen seeds were sown September 25 in each pot, and after the young shoots had reached 8-10cm. they were reduced to 4 per pot, all

of nearly equal height. Some time afterwards the nickel plants appeared to be ahead of the others. Towards the middle of November it became evident that the leaves of the cobalt plants were considerably larger than those of the others. Unfortunately some damage by a caterpillar was done to some leaves of the zinc plant before the insect was noticed, hence further observations of this pot were abandoned. On December 5 the pots received 0.5g. ammonium sulphate and 0.5g. monopotassium phosphate highly diluted, since some of the lower leaves had a yellowish appearence. The plants were harvested on December 23 with the following results:

	Number of leaves.	Length of leaves (cm).	Fresh weight, plant mass.	Fresh weight of roots.	Root in percentage of total yield.	Weight of the fresh leaves(g.)
Ni—plants	41	14,9	31,1 (g.)	11,4 (g.)	36,5%	21,25 (g.)
Co— „	37	16,5	34,8 „	11,7 „	33,6 „	22,6 „
Control	43	13,5	30,1 „	13 „	43,2 „	17,4 „

It will be observed from this table that in the case of cobalt some noticeable increase of the total production took place but this increase of weight related only to the leaves while the weight of the root was smaller than in the control case. In the case of nickel an insignificant increase of the total weight is noticed which was due to the leaves but not to the root. What with safety can be concluded from this table is only that a stimulant action on the growth of the leaves by the action of a small quantity of cobalt nitrate had taken place.

Experiment with Hordeum.

After cutting *Bressica Chinensis* the same pots were manured with 3 grams sodium nitrate, 3 grams potassium carbonate and 4 grams double superphosphate. No fresh doses of the above named metalic salts were given and thus only the amount left after harvesting *Brassica* came here into action. Twenty seeds of barley per pot were sown on January 14.

On February 1 the young shoots were reduced to 10 per pot of nearly equal height. In the beginning of March the cobalt plants were taller than the control plants while the zinc and nickel plants were lower. On March 10 the average length was:

	Average length
Zn plants	36 cm.
Ni „	38 „
Co „	40,5 „
Control	39,5 „

On March 12 the ears of the cobalt plants commenced to appear while those of the zinc plants on the 18th, those of nickel on the 15th and those of the control plants on 13th. These plants were harvested on May 21 and left to dry. The result was as follows:

	Total weight.	Weight of ears.	Weight of grains.	Number of grains.	Weight of straw (air dry g.)
Zn—plants	18,6 g.	6,7 g.	5 g.	135	11,9 g.
Nickel „	23,0 „	9,2 „	7,1 „	181	13,8 „
Cobalt „	25,3	10,2 „	8,2 „	202	15,1 „
Control „	24,0	9,4 „	7,3 „	192	14,6 „

It will be noticed that only with cobalt a stimulant action had taken place and that nickel and still more zinc exerted even in the very small doses present an injurious action on the barley.

Experiment with Pisum.

Twenty seeds of Pea were sown February 16 and the young plants were reduced later on to 4 of equal height in all the pots. In the early period of growth, the nickel plants showed some stimulation and at the beginning of May, also the zinc and cobalt plants in comparison to the control plants. The following observations were made on May 7:

	Average length.
Zn–plants	83,25 cm.
Ni– „	92,5 „
Co „	81,75 „
Control	75,75 „

Growth as well as the flowering period lasted longest with the control plants. The harvest on June 17 yielded the following results:

	Total weight.	Weight of seeds.	Number of branches.	Average length.
Zn–plants	24,5 g.	14 g.	4	120 cm.
Ni– „	27,8 „	14,5 „	4	123,8 „
Co– „	25,5 „	14,3 „	5	118 „
Control	24,5 „	14 „	4	120 „

It will be seen that the stimulating effect of Zn was nil and those of Co and Ni minute and uncertain. In all the 4 cases here described I have observed that in the early period of develompment the nickel plants showed the most favorable growth and from the middle period of development cobalt plants gained headway while the control plants continued its growing and flowering for a longer time than the others.

As a general result, however, it may be inferred that stimulant actions can be exerted in certain cases by small doses of zinc, nickel and cobalt salts, on agricultural plants, but this effect was not considerable in my experiments.

Can Lithium and Cæsium Salts Exert any Stimulant Action on Phænogams?

BY

M. Nakamura.

Lithium belongs to those elements which are present in very small quantities in many soils.[1] It was found also in many plant ashes by *Bunsen* and *Kirchhoff*.[2] Lippmann found it in the ash of the sugar beet, *Truchot* in the ash of tobacco (0.44%). *Tschermak* observed that lithium especially accumulates in the leaves, and that certain plant species take up lithium salts much more easily than others. He further denies any relation between the lithium contents and the degree of development. *Nobbe* (1871) had observed that lithium cannot replace potassium in the plants, and that lithium salts act even poisonously upon buckwheat.[3] With algæ and fungi no injurious action of lithium salts was observed.[4] It remained now to be seen whether lithium salts are capable to exert a stimulating action on phænogams when applied in very small doses.[5] This question seemed to me of sufficient interest to justify some experiments in this direction.

[1] Truchot C. r. 78. 1022.

[2] Ann. Chem. Pharm. Vol. 118 p. 353.

[3] The culture solutions of Nobbe contained 104.2 milligr. Li_2O per liter, in another case 73.8 milligr.

[4] O. Loew, Ein natürliches System der Giftwirkungen. p. 115. Also Journal prakt. Chem. Bd. 36, S. 284 (1887).

[5] According to *Ono* lithium nitrate can act as a moderate stimulant of growth on algae and fungi (Journal College of Science, Tokyo, vol. 13 p. 165(1900)):—Thus $LiNO_3$ in a dilution of $\frac{1}{32} \times 10^{-4}$ increased the growth of *Protococcus* in 24 days, from 0.010 g. in the control case to 0.020 g. and the growth of *Aspergillus niger* was stimulated by $\frac{1}{32} \times 10^{-2}$ from 0.300 g. in the control flask to 0.408 g. in 17 days. *Richards* observed stimulant action of 1% Li Cl in the culture solution on fungi (Pringsheims Jahrb. wiss. Bot. Vol. 30, p. 665; 1897).

The soil serving for my experiments came from our College Farm at Komaba and was manured with 1 g. Na NO_3, 1 g. K_2SO_4, 0.5 g. KCl, 0,5 g. $(NH_4)_2$ SO_4 and 1,2 g. double superphosphate per kilo. To one pot was added 10 milligr. lithium carbonate per kilo soil, to the second 100 milligr. per kilo while the third served for the control plants. The pots contained 8 kilo soil each and were kept in the greenhouse.

Experiment with Barley.

Twenty grains of barley were sown January 16 and the young plants reduced later on to five of equal hight in all the pots. At the beginning of May some difference in development was observed and the measurements were as follows:

	Average length	Number of branches
100 mg. Li_2CO_3 per kilo soil	72 cm.	24
10 „ „ „	75 „	24
Control	74 „	22

On May 27 the measurements were as follows:

	Average length	Number of branches
100 mg. Li CO_3 per kilo soil	76.0 cm.	27
10 „ „ „	81 „	24
Control	75.1 „	23

The plants were cut June 13 with the following result:

	Total weight	Weight of grains	Number of grains	Weight of each 100 grains	Average length of branches	Number of branches
100 mg. per kilo soil	104 g.	44.7 g.	887	5.28 g.	76.3 cm.	27
10 mg. per kilo soil	114 „	43.5 „	808	5.38 „	81.5 „	24
Control	97 „	38.5 „	787	4.89 „	75.3 „	23

Experiment with Pea.

The conditions were here essentially the same as in the first experiment. Twenty grains of pea were sown January 28 and the young plants reduced later on to four of equal height in all the pots. In the early period of development the control plants showed the best growth and also showed some flower several days earlier than the lithium plants.

Measurements were made on May 5 with the following results:

	Average length of branches	Number of branches	Number of flowers
100 mg. Li_2CO_3 per kilo soil	86 cm.	5	1
10 „ „ „	98.3 „	4	3
Contro	101.5 „	4	6

After that time the lithium plants showed a more vigorous development than the control plants.

The plants were harvested June 15.

Air dry plants.

	Total Weight	Weight of seeds	Number of seeds	Number of branches
100 mg.	65 g.	30.5 g.	147	5
10 mg.	61 „	31.2 „	142	4
Control	56 „	29.5 „	140	4

Both experiments show that *lithium carbonate can exert a slight stimulant action.*

Under the conditions just described an experiment with upland rice was carried out to test the influence of cæsium on the development. Pot A received 10 Milligr. cæsium chlorid per kilo soil, Pot B 100 Milligr. while Pot C served as control. Young plants of equal height were selected from the seed bed and planted into the pots a five in each, at the beginning of June. At the time of flowering it was evident that the plants in B exceeded the others as to height. The plants were cut October 2. In pot A no branches were developed in B one branch with yet unripe seed, while in C one branch with ripe seed.

The measurements gave the following figures:

	A	B	C
	100 cm.	109 cm.	109 cm.
	101 „	112 „	101 „
	103 „	113 „	108 „
	105 „	114 „	91 „
	105 „	101 „	84 „
Average height.	103 „	110 „	99 „

The height of the branch in B was =58 cm; in C=75 cm. The total production of ripe seed, weighed in the air dry state was:

A	B	C
13.1 g.	14.6 g.	13.7 g.

It can therefore be inferred, that cæsium chlorid at the rate of 100 mg. per kilo soil had exerted a moderate stimulating effect, especially noticeable in the height of the plants.

On the Stimulating Effect of Iodine and Fluorine Compounds on Agricultural Plants II.

BY

K. Aso and S. Suzuki.

In former Bulletins of this College, a field experiment with radish and pot experiments with oats and pea were described which showed the stimulating effect of small doses of sodium fluorid and potassium iodid. We have continued these investigations, upland rice serving now for the test. Fife plots were selected, each of these had ten square meter and received as manure 100 g. double superphosphate, 200 g. ammonium sulfate and 150 g. wood ash, the last mentioned salt being ploughed into the soil one day later than the former. The seed to the amount of 47.5 g. per plot was sown April 30. Plot I received 0.08 g. Na F, II 0.8 g. Na F, III 0.025 g. KI, IV 0.25 g. KI, and V served as control plot. The solutions were applied in high dilution. On June 2, each plot received as top-dressing 50 g. sodium nitrate and 50 g. monopotassium phosphate. The plants on the fluorine plot surpassed gradually the others somewhat in height. A damage done by a typhoon proved to be insignificant.

The plants were cut Sept. 8 and left to dry in the glasshouse. The weight was as follows:

Ratio per ha	Weight of seeds unhusked, g.	Weight of straw air-dry, g.
80 g. Sodium fluorid	2465	1885
800 g. „ „	2090	1800
25 g. Potassium iodid	2300	1900
250 g. „ „	2000	1810
Control	1970	1920

A stimulating effect on the seed production by sodium fluorid and potassium iodid respectively is here evident with the smaller doses of 80 g. sodium fluorid and 25 g. potassium iodid per ha, while the ten times higher doses failed to give a decisive result.

On the Treatment of Crops by Stimulating Compounds.

BY

Oscar Loew.

At the International Congress of Applied Chemistry in Berlin, June 1903, Professor *Gabriel Bertrand* from the Institut *Pasteur* in Paris read a paper: "Les Engrais Complémentaires" in which was pointed out that, as to mineral manures, almost exclusively compounds of potassa, phosphoric acid and nitrogen are paid attention to, while there exist rarer elements which occur only in exceedingly small amounts in the plants but may nevertheless be of a certain physiological signification, leading to an increase of the harvest. *Bertrand* proposes to call such compounds supplementary manures (engrais complémentaires).

It may therefore not be out of place to call attention to the fact that professors and graduates of this College have during the last three years studied the influence of small doses of various compounds upon the growth of plants and yield of crops and published a number of papers on this subject.[1] A short survey of the results obtained may here be in order since some observations are of theoretical interest and some promise even to become of practical value. In the first place such elements were considered which occur in small doses widespread in the soil whence they pass into the plants and through them into the animals. These elements are manganese, fluorine and iodine. The manganese content of vegetable and animal organs,

[1] These Bulletins Vol. 5, No. 2 and 4; also this number. A part of the former experiments was also reviewed by the writer in the Landw. Jahrb. 1903, Heft. 3.

the fluorine content of bones and teeth, the iodine content of the thyroid gland are well known facts.[1]

Although according to *Gautier* and to *Bertrand* slight traces of arsenic also occur in animal organs which must have received it through plants from the soil, we did not take this element into consideration. Arsenious salts in a dilution of 1:300000 are still very poisonous for phænogams (*Nobbe*). Arsenates, it is true, are much weaker poisons for plants, but nevertheless also strong poisons for warmblooded animals. The use of arsenic compounds in any form should not be tolerated in agricultural operations. In the United States poison cases were caused by cabbage that had been dusted with Paris green in order to kill the adhering caterpillars. It is true that most of the stimulating compounds are poisons in higher concentration, but the dangers are nowhere as great as with arsenic compounds.[2]

In the second place also compounds of such elements were tested which occur occasionally in traces in the soil and of which no occurrence in animal organs is reported, as boron, lithium, rubidium and cæsium compounds.[3] The high price of the salts of these elements would forbid their practical application.

In the third place compounds of elements were tested which are confined to certain localities of relatively rare occurrence, as zinc, nickel, chromium, cobalt, uranium, thorium, vanadium, cerium. (Bromine was tested on

[1] *Gley* and *Bourcet* found iodine in the blood (0.013—0.112 milligr. per Liter). The occurrence of iodine compounds in mineral springs, in some rocks and minerals, as, e.g., phosphorite and in coal are well known. Traces of them occur also in the Chili salpeter. As to fluorine it was shown by *Tamann* to occur also in eggalbumen, milk and blood (about 1 milligr. in 100 parts of fresh substance. *Nicle's* found traces of fluorine in various animal organs as early as 1856. The origin of the traces of fluorine in the soil is the apatite occurring in varolus rocks.

Also alumina occurs in plants frequently. A stimulating action of alumina was thus for not distinctly rerognized by us, but the experiments will be continued with larger doses of aluminium salts.

[2] Fungi are not quite so sensitive towards arsenious acid, as green plants. According to *Orlowski* 0.001—0.01% sodium arsenite stimulates growth of *Asperfillus niger*, larger quantities act retarding, still larger as strong poison.

[3] It is of some interest to note that *Dieulafait* has discovered traces of lithum, rubidium and boron in the crude Chili salpeter (Compt. rend. **48**, p. 1545). Traces of these and of cæsium and titan were repeatedly observed in plant ashes; also of vanadium in one case by *O. von Lippmann*.

account of its occurrence in sea weeds and for the sake of comparison with fluorine and iodine). All such compounds are foreign to agricultural soils and should therefore be excluded from practical application as stimulants.

For the convenience of the reader some of the observations made by *Nagaoka, Aso, S. Suzuki, Nakamura* and the writer have been embodied in the following Table:

Stimulating Compound.			Remarks.
Name.	Milligrams per kilo soil.	Amount per hectare.	
Lithium carbonate	10—100		Pot experiment with pea and barley.
Rubidium chlorid	200		Pot experiment with barley.
Cæsium chlorid	100		„ „ rice.
Uranium nitrate	5		„ „ pea and oats.
Manganous sulphate $MnSO_4+4$ aq.....	24		Pot experiment with pea.
„ „ „		77 kilo	Field experiment with rice. [Mn_2O_3 = 25 kilo per ha]
Manganous chlorid $MnCl_2+4$ aq.		63 kilo	Field experiment with upland rice.
Borax.....	1—5		Pot experiment with spinach and pea.
Bromid of Potassium	10		Pot experiment with beans and rice.
Iodid of Potassium	0.26		Pot experiment with oats.
„ „ „		25 grams	Field experiment with radish and upland rice.
Fluorid of Sodium	2.6		Pot experiment with pea.
„ „ „		80—140 grams	Field experiment with radish and upland rice.

The degree of the poisonous character of compounds does not always correspond to the intensity of the stimulating action, exerted when highy diluted, and also the zone of indifference, i.e. that special degree of dilution

at which the stimulating effect and the injurious influence balance each other, is of very different width, it seems e. g. larger with fluorid of sodium than with borax. Vanadium sulphate, poisonous at 0.1% for plants in water culture, still exerted a depressing influence at a rate of 10 milligrams per kilo soil. Borax acted in this dose also very injuriously but at 1–5 milligrams it exerted a weak stimulating action. Chromic alum exerted a poisonous action in a dilution of 0.1% upon seedling of pea, but had in higher dilutions neither an injurious nor a stimulating effect.[1]

Of some interest is the stimulating effect of lithium, rubidium and cæsium compounds in doses of 0.01—0.2g per kilo soil, since it recalls the beneficial actions of sodium salts observed by many authors, and studied especially by *Wagner*. According to *Doll*[2] a maximal yield of barley can only be expected by the joint application of potassium and sodium salts.

The question how the stimulant action is to be explained can at present not be answered positively, although in some cases certain views find some support. It is thus, e. g., very probable that manganese salts act beneficially by enhancing the action of the oxidizing enzyms in changing noxious by-products of metabolism by partial oxidation.[3] The fact that potassium bromid, manganese, and uranium compounds exert a stimulating effect on phænogams but not on fungi, while sodium fluorid acts stimulating on both groups of the vegetable kingdom, shows plainly that there exist various causes for the phenomenon of stimulation.

As to zinc salts a stimulating effect was observed with a fungus (*Aspergillus*) as well as with phænogams (*Raulin, Kanda, Nakamura*), but the fungi appear to behave not all alike, since *Coupin* observed no stimulating effect of zinc salts on *Sterigmatocytis nigra*.[4]

[1] A soil containing 1.6% chromic oxid occurs in the island of Adaman. The coffee plantations on that soil are reported to be normal. (J. B. f. Agr. Chem. 1891).

[2] Centralbl. f. Agric. Chem. **32**, p. 13.

[3] Cf. These Bulletins, vol. V, No. 2, and Flora 1902 p. 264.

[4] Compt. rend. Febr. 1903. In experimenting with mould fungi quite a number of flasks, not only one or two, should be observed, since often widely different weights of fungus growth under apparently equal conditions are obtained.

The reason for testing uranium and thorium salts was their showing radioactive properties. Radioactivity has a powerful influence on animal tissues and on bacteria and some influence was probable to be exerted also on phænogams. Both those compounds, however, differ widely in their effects on plants, uranium salts being highly poisonous, thorium salts not, uranium salts stimulate further in much smaller doses as do thorium salts. Uranium salts also can produce under the influence of light certain chemical changes, which thorium salts are incapable of. These specific changes are not connected with the reduction of uranic to uranous compounds by organic substances under the influence of light. They consist in splitting off one carboxylgroup from the molecule of a bibasic acid. Oxalic acid becomes thus formic acid; succinic acid changes to propionic, pyrotartaric to isobutyric,[1] glutaric to butyric acid. Mesaconic acid seems to yield crotonic acid. Itaconic, tartaric, citric and suberonic acid are but very slowly changed, if at all. Asparagin and peptone remain also apparently unchanged, while parabanic acid is easily changed to ammonia, formic and carbonic acid. Thus the view of the writer seems to have some support that traces of uranium compounds that had passed into the leaves might enhance the transformation of light into chemical energy.

Several experiments were made with uranium salts. In the successful pot experiments with pea and oats, the highly diluted uranium nitrate was applied as top-dressing in six doses. The increase compared with the control plants was in regard to the seeds 1.27 fold with the pea and 1.25 fold with oats. A field experiment with upland rice however was not successful, the yield being too little above that of the control plot. The uranyl nitrate was here applied together with the manure and not as top-dressing (6 g. for 30 square meter) and thus became, as tertiary phosphate, gradually entirely unavailable for the roots.[2]

[1] Seekamp, Ann. Chem. Pharm, vol 133 [1865]. He added to a 5% solution of succinic acid 1 percent uranious succinate and exposed this mixture to the direct sun light.

[2] An unfavorable circumstance was here probably also the application of potassa as carbonate (woodash).

A joint application of two stimulants proved in some cases advantageous[1] in others, however, not. A report on these experiments will follow later on.

The question might be raised whether the seeds of plants grown under stimulating influence would yield again normal plants. In this regard quite a series of tests were made, all of which answered in the affirmative.

Of all the stimulants observed only compounds of manganese, fluorine and iodine come seriously into consideration for practical agriculture. Besides these also the ferrous compounds deserve attention, which apparently act not only as nutritive material inasmuch they render possible the formation of chlorophyll but also exert some kind of stimulating action. The observation of *Molisch* on the relation between iron and fungi shows that iron is not only concerned in chlorophyll production.[2] Indeed iron like manganese was found repeatedly in the ash of nucleoproteids (*Stoklasa, U. Suzuki, Aso*) and of oxidizing enzyms (*Bertrand, Lepinois, Sarthou, Spitzer, Sieber*).

The action of ferrous sulphate upon crops has often been an object of observation. Some authors reported a favorable action, some an unfavorable one; others again inferred that there was no influence whatever. The effects depend of course very much upon the quantity applied. In one case fully 0.1% was added to the soil in order to prove its injurious influence. This is a very large dose.[3] The effects also depend upon the quantity of easily absorbable iron already present in a soil; a further moderate addition may prove of no avail at all when the soil is rich in easily available iron compounds. Our own observations showed that doses of 25—50 Milligrams ferrous sulphate per kilo soil can exert some stimulating action even on a soil that contains enough easily available iron for the chlorophyll formation

[1] This was, e. g., the case in the joint application of ferrous and manganous sulfates.

[2] *W. Benecke* observed with a moss (*Lunaria cruciata*) that the formation of rhizoides was at first retarded by solutions containing 0.0004% ferrous sulfate, later on, however, enhanced (Botan. Zeitg. 1903, Heft. 2.).

[3] The occasional occurrence of ferrous salts or ferrous sulphid in swampy grounds is only a sign of wanting aeration but not the original cause for the inferiority of such soils. *Griffiths* observed very good results by green vitriol at the rate of 60—125 kilo per ha. This amount may be for certain crops too high.

of crops. Whether this effect is due to the state of the monoxid, while in the soil iron was present as sequioxid or whether it is due to the much finer state of division of the applied iron compound we are at present not yet prepared to decide.[1] It may here be mentioned that ferrous sulfate is capable of certain catalytic powers. Thus a slight trace of it suffices to bring hydrogen peroxid into action with potassium iodid, whereby the iodine liberated can at once be recognized by the intense blue color produced with starch paste (*Schönbein's* reaction for hydrogen peroxid). In this way still 0.0000001 grams iron in the state of ferrous salt can be recognized.[2]

In some of our experiments the effect of small doses of ferrous sulphate was compared in absence and in presence of manganous sulphate. A pot-experiment with oats may here be described. Four pots each holding 2300g. air dry soil and manured each with 3 gram sodium nitrate, 3 g. potassium carbonate and 4.6 g. double superphosphate received 15 seeds on February 21. After the shoots had reached 12—15 cm. their number was reduced to 5 per pot, taking care that these remaining plants were all of equal size. While one pot served as control, the others were watered with highly diluted solutions of ferrous and manganous sulphate six times until the flowering period was passed. All pots were treated alike as to watering, exposure to light, etc. The plants were cut July 6, and left to become air-dry. The final result was:

	$FeSO_4$ = 0.126 g.	$FeSO_4$ = 0.06 g. $MnSO_4$ = 0.06 g.	$FeSO_4$ = 0.012 g. $MnSO_4$ = 0.126 g.	Control
Number of stalks	12	8	15	9
Grains, unhusked	25.7 g.	23.1 g.	27.8 g.	21.4 g.
Straw	48.1 g.	46.4 g.	51.0 g.	45.2 g.

[1] The state of division has a very great influence also with other compounds. Thus precipitated magnesium carbonate (basic) has a much more injurious influence on plant growth than an equal dose of even very finely powdered magnesite. Slaked lime yields a much more finely divided carbonate (in presence of sufficient water), than can be obtained by pulverizing lime stone.

[2] *O. Meyer*, Chemiker Zeitung 1903, No. 52. See further *Schönbein*, Journ. f. prakt. Chem. 1860, p. 66. Also *Schone*, Ann. Chem. Pharm. 1879, p. 232; finally D. Chem. Ges. Ber. 1901, p. 2479.

Some beneficial effect of ferrous sulphate was here doubtless exerted, the effect of the same amount manganous sulphate in presence of some ferrous sulphate was, however, more marked.[1]

Another experiment with tobacco plants, grown in pots from seed from Java, may here be mentioned. Two plants, each measuring 44 cm. received, Oct. 6th, 0.3 g. manganous sulpate ($MnSO_4+4$ aq.)+0.2 g. ferrous sulphate ($FeSO_4+7$ aq.) dissolved in 100 cc. water; two other plants of 48 and 47 cm. height, 0.3 g. manganous sulphate alone; two others, each of 43 cm., 0.2 g. ferrous sulphate, while two, of 45 and 43 cm. height, served as control-plants. The longest leaves measured 32-36 cm. The soil had been manured with farmyard manure; each pot received, Oct. 15, a further addition of 0.2 g. ammonium sulphate. During the month of November considerable differences in growth became easily noticeable. On Dec. 23 the plants were photographed; this photograph is reproduced on Plate XV. They were cut the same day with the following result:

	Mn + Fe	Mn	Fe	Control
Height of the plants, cm.	160 135	155 134	153 122	115 109
Percentage of increase in height.	260 207	223 185	209 184	155 153
Number of flowers	15	4	14	—
Number of buds	48	46	41	—
Number of dead leaves	7 6	7 9	10 7	7 8
Number of living leaves from 12 cm. upwards	17 19	21 18	19 16	17 17
Weight of the fresh leaves	84.2	85.5	75.2	59.5

[1] The application of ferrous sulphate and manganous sulphate on the farms would be very cheap, since both there salts can be directly applied in the raw, unpurified state. Crude manganous chlorid $MnCl_2+4$ aq. is obtained as a by-product of the manufacture of bleaching powder and costs in Japan only 3 yen per 100 pounds. In Germany its price is more than double as high (16 Mark).

It will be seen that ferrous as well as manganese sulphate had exerted a stimulating action and that the best effect resulted also here by the joint application of both.

Manganous sulphate and chlorid exert a stimulating effect on various crops,[1] but the degree varies according to circumstances. Not only the mode of application but also the manures used influence the result. Repeated application of highly diluted solutions in the form of top-dressing are more favorable than a single application of the same total amount of manganese salt at the time of manuring the soil, before the seed is sown. All conditions further which change the manganous salt soon into manganic oxid or tertiary manganic phosphate appear to depress also the availability of the manganese for the roots. It seems highly probable that potassa applied as carbonate or in the form of woodash will in this respect act unfavorably while in the from of sulphate not. In pot experiments the application of phosphoric acid in the form of secondary sodium phosphate acted less favorable than in the form of double superphosphate. Thus in a pot-experiment with pea the harvest was increased under the influence of manganese 50% in straw and 25% in seed compared with the equally well manured control pot, while in an experiment with buckwheat made under the unfavorable conditions mentioned, no increase was produced.[2] A most remarkable result was obtained by Prof. Honda and myself with young Cryptomeria trees, which received manganese sulphate as top-dressing in monthly doses, winter excepted, for one year and a half. The organic production was hereby doubled compared with the control trees.

It is further evident that fields which served repeatedly for raising crops under the influence of stimulating agencies would sooner be exhausted than others. Hence a moderate increase of manure will be needed. The following experiment seems to furnish an example: Two plots of 64 sq. meter were manured each with 640 g. double superphosphate, 1000 g. ammonium

[1] The susceptibility of different plant families toward stimulants seems to differ. Further observations will be made here to decide this point.

[2] Injurious effects of manganese in comparatively large quantities have thus far only be observed with watercultures (Landw. Vers. Stat. vol 8, p. 128 and **73** pp. 69 and 218).

sulphate and 1000 g. wood ash (added 8 days later). Seed of radish was sown Sept. 1. (1902). After the plants had reached a height of 15–25 cm. one plot received a top dressing of 94 g. cryst. manganous sulphate (=64 g. anhydrous Mn SO_4) dissolved in 20 Liters of water. The superfluous plants were removed Oct. 8, leaving on each plot an equal number of equally well developed plants. A storm, however, injured a number of plants on both plots, hence only the best developed roots were compared. The height of the plants at the time of harvesting, Dec. 17, did not show any marked difference. The result was:

	Manganese plot	Control plot
Total plant mass (fresh weight).....	57.68 kilo	51.18 kilo
The 20 largest roots weighed	11.1 „	8.9 „

The same plot served now for a growth of potatoes. Amount and kind of manure was the same as in the former case, but manganese was not applied this time, in order to observe, whether some action would still be exerted by the manganese left from the first application. On March 23 (1903) 120 potatoes of equal size were planted on each plot, 10 potatoes in each row. The soil was cultivated several time and weeds removed. In the beginning of July some damage was done by a storm. The harvest, on July 16 yielded:

	Manganese plot	Control plot
Potatoes	17 kilo	15.6 kilo

It appears therefore that some effect of the manganese left was exerted, but the difference is not of very decisive magnitude.

The plots received now farmyard manure at the rate of 8 tons per ha and cryst. Manganese chlorid at the rate of 10 kilo per ha, and served for a growth of millet, of which 110 g. seed were sown on July 24. The harvest on Oct. 5 yielded:

	Manganese plot	Control plot
Total plant mass air-dry	25.1 kilo	26.0 kilo

Here several circumstances probably united to prevent the stimulating action of the manganese. In the first place the rate of manganese chlorid applied was rather low, in the second place there were less nutrients left after the stimulated crops of radish and potatoes had been grown than in the control plot, and in the third place the manganese was not applied as top-dressing but in conjunction with the general manure. It goes without saying that in the form of top-dressing the same amount of manganese must be more effective under otherwise equal conditions than when the distribution is made uniform through the whole soil. An exceedingly favorable result obtained with mustard on the one hand and a poor result with cabbage on the other hand support this inference.

Experiments with clover and maize have further shown that a favorable action of manganese cannot be expected when the manuring is imperfect. In these cases ammonium sulphate alone at the rate of 500 kilo per ha was applied, since some potassa and phosphate was supposed to be left from previous crop.

The manganese plot that had received manganese sulfate at the rate of 10 kilo per ha yielded here the same poor harvest as the control plot. The cobs of the maize on the manganese plot, however, showed in average a little higher weight than those on the control plot, viz 413 g. versus 391 g.

Soils which year after year are manured with farmyard manure are unvoluntarily gradually enriched with manganese compounds in finely divided condition, since the dung of cattle and horses contains almost the total quantity of manganese contained in the original fodder. Let us calculate for a given case how much manganous oxid would thus be supplied to one hectare manured with 36 tons of farmyard manure. *A. Emmerling* and *R. Wagner*[1] determined in a sample of meadow hay the amount of manganous oxid to 0.2% of the ash; the ash itself amounted to 8.86% of the dry matter of the hay. The farmyard manure (with 80% H_2O) from this hay would contain in 36 tons =7200 kilo dry matter and in this =1.26 kilo Mn O hence after 10 years the soil receives 12.6 kilo Mn O corresponding to 32.4 kilo

[1] Wolff's Tables of Plant Ashes. II, p. 28.

crystallized manganous chlorid $Mn\,Cl_2 + 4$ aq. In beets[1] was found 13.95% ash in which 0,025% $Mn_2\,O_3$ and 0.048% $Fe_2\,O_3$; in clover 9.17% ash in which again 0.27% Mn O and 1.51% $Fe_2\,O_3$. These numbers, of course, vary according to the chemical composition of the soils but in general it may be inferred that the beneficial action of application of manganese salts will be more decisive where mineral manures are used than on soils that are continuously manured with dung. Various facts render the farmyard manure often superior to mineral manures,[2] as improvement of the mechanical condition of certain soils, the introduction of a rich bacterial flora into the soil, etc. One of the favorable circumstances may also be the presence of finely divided manganese and iron compounds.

Plants which grow on soils containing much manganese in available condition will hardly be benefited by application of further doses of manganese salts. The manganese content of soils differs considerably. Relatively small differences are shown in the analyses of four soils mentioned in *Wolffs* Tables.[3] Concentrated hydrochloric acid extracted at the ordinary temperature from:

	$Mn_2\,O_4$	$Fe_2\,O_3$
Loamy soil	0.135%	2.096%
Clay „	0.180 „	3.173 „
Sandy „	0.083 „	1.039 „
Humus „	0.042 „	0.406 „

In the fine earth of a soil from Barbados[4] 0.1% $Mn_2\,O_4$, soluble in hydrochloric acid was found, corresponding to 38 kilo $Mn_3\,O_4$ per ha to the depth of 24 cm.

[1] Ibid., p. 43 and 37.

[2] Recently again *Schneidewind* reported from the experimental farm at Lauchstädt that the root crops yield a maximal harvest only when farmyard manure is applied in addition to mineral manures. Such observations have been made also in regard to tobacco in America.

[3] Vol II, p. 16.

[4] Report of the Barbados Experiment Station 1901.

It was mentioned above that also iodine and fluorine compounds might sometimes be used as stimulants in practical agriculture. Some further remarks are therefore in order. As to iodide of potassium it acts poisonously upon phaenogams in waterculture even in a dilution of 0.002 per cent. *Voelker*[1] observed in a field experiment that a top-dressing at the rate of one half centiweight per acre, corresponding to 62.2 kilo per ha injured wheat and barley. This quantity, however, seems extraordinary great when compared with the amount that caused stimulation in our experiments, namely 25 grams per ha! This quantity can be increased to 250 grams per ha without fear of danger, but a further essential increase should be avoided. Hence a dose of 25 g. per ha may be applied for ten consecutive years; if it is taken into consideration, however, that a part is absorbed by the plants and another passes away in the drainage waters, the number of years might be doubled. But after this period a pause of several years should follow, during which the use of potassium iodid is suspended. The degree of stimulation was in some experiments of *S. Suzuki* considerable[1] The dose at the rate of 25 gram per ha produced an increase in the weight of radish of 67% compared with that obtained on the control plot and with upland rice an increase of 16% in grains. At the rate of 250 g. per ha the increase in the weight of radish was 31% while there was no increase in seed production with rice.

As to fluorine compounds of potassium or sodium it must be kept in mind that these are in certain respects still more poisonous than potassium or sodium iodid. While the latter exerts a high degree of poisonous action on all plants with an acid cell sap and but a weak one on objects with a neutral cell sap, as e. g. certain algae, those fluorids are very poisonous for all kinds of vegetable objects independent of the reaction of the cell sap or culture. A few observations may here be mentioned. Algae, such as *Spirogyra* and *Mesocarpus* are killed within 15 minutes in a 1 per cent solu-

[1] In pot experiments stimulation was observed at 0.26 and 2.6 milligrams per kilo soil with oats and pea, while at 26 milligrams a depression resulted with rice.

tion of sodium fluorid, the chlorophyll bands retract their lobes, the nucleus contracts and soon afterwards the cytoplasm recedes from the cellulose wall. In a solution of 0.1% filaments of algae die within 24 hours. Diatoms and monadines become motionless in a 0.1% solution within 12 minutes. Some monadines recover their power of motion but this is only of short duration, since after 35 minutes no trace of motion reappears. If 1 cc. of a 1 per cent solution of sodium fluorid be added to 99 cc. of culture water containing numerous forms of minute organisms, infusoria and diatoms are killed within 2 hours, while some monadines[1] still were seen alive after this time. At 0.05 per cent sodium fluorid injures the germinating power of seed, while in dilutions of 0.0001% to 0.001% it can stimulate growth of phænogams in water culture.

At 0.001% it prevents the action of lactic acid bacilli (*Effront*) and at 0.15 g. per kilo body weight it proves fatal for animals (*Tappeiner*).

Attention must here be drawn to the socalled *Wiborg's* Phosphate which contains fully 1 percent fluorine and is recommended for agricultural purposes. Since phosphates are often applied in large doses as manures, a highly injurious amount of fluorine would soon be accumulated in the soil. This phosphate is manufactured in Sweden by fusing apatite with sodium hydrate and consists chiefly of a sodium—calcium silico—phosphate.[2] Calcium phosphate is now-a-days also frequently added to the food of young hogs in order to promote the formation of bone. There occur, however, in commerce phosphatic preparations containing fluorids which have caused the death of the animals, as *Emmerling* has shown recently.[3]

The stimulating effects of small doses of sodium fluorid, viz 80—140 g. per ha have been quite considerable in the experiments of K. Aso, who has observed further that even a dose of 800 g. per ha does not yet act injuriously, although the stimulating effect has decreased, or almost vanished, showing that a further augmentation of the dose would not be advisable. A part of the sodium fluorid or all may pass into the but little soluble calcium fluorid, which dissolves in 26923 parts of water at 15.° a circumstance that tends to diminish somewhat the evil effects of accumulation. The stimulation of seed production with upland rice amounted to fully 25 percent on a plot that had received sodium fluorid at the rate of 80 g. per ha.

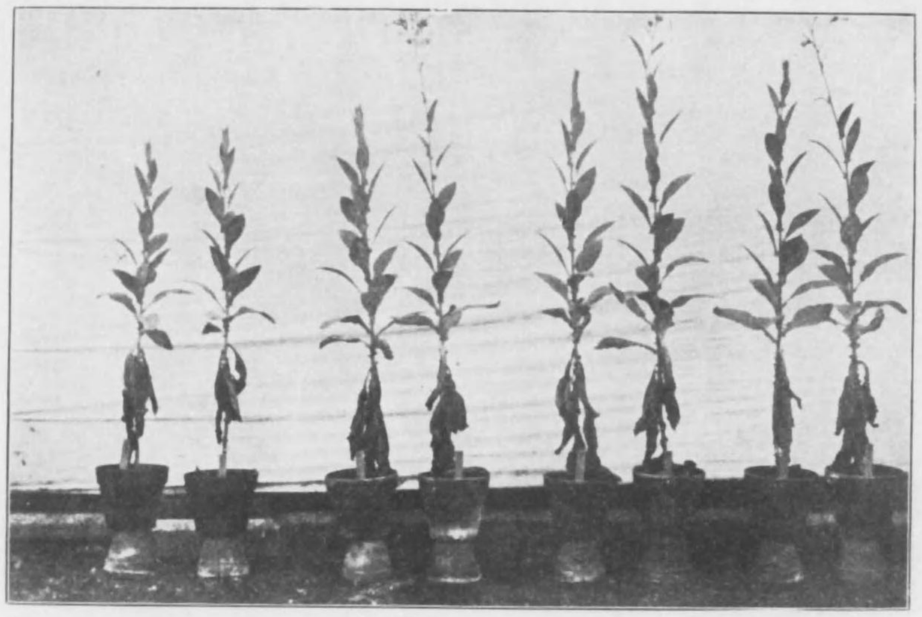
[1] The monadines are also in other regards of an unusual resistance power which thus far is not satisfactorily explained.

[2] *Wilborg's* phosphate was recently reported in the Chemiker Zeitung to have the following composition; $P_2O_5 = 22\%$; $SiO_2 = 16\%$; $MgO + CaO = 35\%$; $Fe_2O_3 = 6\%$; $Al_2O_3 = 2\%$; $K_2O + Na_2O = 18\%$; Fluorine = 1%.

[3] Centralbl. f. Agr. Chem. June 1903.

It will be noticed that the application of iodids and fluorids as stimulants requires a certain attention. Since perhaps the majority of farmers are averse to pay sufficient attention to the laws of nature, it may be recommended to restrict the application of stimulants to the manganous salts, since there are no dangers to be feared from their continuous application and any excess gradually turns into not readily available insoluble manganic compounds. They should be applied only in top-dressing—in several doses if possible—at the rate of about 25 kilo per ha—in high dilution and in addition of ferrous sulphate at the rate of about 20 kilo per ha.[1]

[1] The ferrous sulphate should be dissolved in cold, not in hot water in order to avoid oxidation and decomposition with formation of basic ferric sulphate.—Manganese as sulphate would be further preferable to the chlorid in many cases.



Tobacco plants under the stimulating action of ferrous and manganous sulphate.

On the Action of Sodium Nitro-prussid upon Plants.

BY

Rana Bahadur,

from Nepal, India.

The highly poisonous character of nitro-prussid of sodium for vertebrate animals was recently demonstrated by *Fonzes-Diacon* and *Carquet.*[1]

It seemed of some interest to test whether this salt would be also a strong poison for the lowest animal organisms and for the plants.

I have observed that infusoria, crustacea and worms were dead after one hour and half in a 0.1% solution of sodium nitroprussid; only some monadines were still alive. Diatoms also were killed after a short time in that solution. Filaments of *Nitella* were dead after an hour and hal in the 1.% solution. Young barley and buckwheat plants placed in the 0.1% solution were dead after 18 hours, the leaves having completely lost their turgor and being partially dried up. Leaves of radish and mulberry and branches of *Polygonum aviculare* were also found much affected in that time, the leaves being more or less withered. The leaves of cherry looked more or less brown when held against the light, while the control leaves were perfectly normal.

Since, however, the solution of nitro-prussid of sodium decomposes in direct sunlight and more slowly also in diffused daylight, with the production of prussian blue and prussic acid,[2] it may be objected that it is the prussic acid formed by this decomposition which killed the organisms.

[1] Chem-Centrbl. 1903, p. 519.

[2] The character'stic odor of this acid is very soon noticed with these solutions.

I have observed that one of the products of decomposition of this salt by sunlight is nitrous acid. Hence the decomposition of nitro prussid of sodium might be represented by the following equation:—

$$Fe(CN)_5 NO Na_2 + H_2O = NOO Na + Na CN + Fe(CN)_2 + 2 HCN$$

On account of this decomposition all further experiments were now carried on in darkness. Young barley plants were placed in 0.01%, 0.1%, 1% solutions respectively, and kept in darkness. In the 0.01% solution of sodium nitro-prussid, the plants were not killed even after three days; while the plants in 0.1% solution commenced after twenty two hours to dry up slowly from the tips downwards. (This is quite different from the observation when the barley was kept in daylight, in which latter case the plant completely lost its turgor and dried up within eighteen hours, and finally the plants in 1% solution were killed within twenty two hours).

Further to a culture water containing numerous lower organisms 0.01% and 0.1% respectively, of sodium nitro-prussid was added and the flasks kept also in darkness. The microscopical examination of the sediment after twenty-three hours showed that diatoms, flagellata and infusoria were still alive, also the ostracodes and small nematodes were seen in their usual motions. Hence it may be concluded that nitro prussid of sodium is in a dilution of 0.1% not injurious for lower organisms, provided the daylight be excluded and thus the production of prussic acid from the salt be avoided.

Some experiments were also made with mould fungi and bacteria. In the case of mould fungi, sodium acetate 0.6% was the organic nutrient while ammonium sulphate, monopotassium phosphate, and magnesium sulphate each 0.1%, formed the mineral nutrients. In the experiment with bacteria, meat extract served as nutrient. In the former case 1% in the latter 0.5% of nitroprussid were added and the solutions after infection with *Penicillium glaucum* and *Bac. pyocaneus* respectively, were placed in darkness. After about one week *Penicillium* was developed about equally well as in the control case. As to the bacteria, the growth was noticed one day sooner in the control case than in the chief flasks which, however, showed after one week, a luxurient development.

Conclusion.

Nitroprussid of sodium is a comparatively weak poison for lower animal organisms and green plants, and no poison for fungi, provided the daylight is excluded.

Daylight decomposes the salt with the production of prussic acid and nitrous acid. Such a decomposition probably takes place also in the higher animal organism which would account for the highly poisonous character of that salt for the vertebrate animals.

On the Behavior of Guanidine to Plants.

BY

I. Kawakita.

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1. Can guanidine serve as nutrient for fungi?

It has been asserted in a paper published some years ago that guanidine may serve as source of carbon in the development of *Aspergillus niger*. This seemed however exceedingly improbable since guanidine yields by hydrolysis carbondioxid and ammonia and there is no hydrogen atom in direct combination with the carbon. It is most closely related to urea which also is incapable to serve as source of carbon in the nutrition of fungi. Both compounds, however, are good sources of nitrogen for various fungi. In order to test whether really guanidine can serve not merely as source of nitrogen but also as source of carbon, I prepared the following solution:

Water	100 c.c
Guanidine hydrochlorid	1. gr
Monopotassium phosphate	0.1 gr
Magnesium sulphate	0.1 gr

Two flasks containing this solution were after sterilization infected with spores of *Aspergillus niger*. For comparison a control flask containing glycocoll in place of guanidine was also infected. After 4 weeks there was no trace of development in the guanidine flasks, while in the control flask there was growth. The same result was obtained with spores of *Penicillium glaucum*. Even *Bacillus methylicus*, which can utilize such poor nutrients as sodium formiate, refused to grow in the above solutious after they were neutralised. Hence guanidine canf not serve as a source of carbon for fungi. However after addition o-

0.1% glucose, development of various fungi, as Aspergillus niger and Bacillus methylicus took place readily.

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2. Can guanidine serve as source of nitrogen for *Phænogams?*

Since Sawa[1] had observed that urea in moderate quantity can exert a poisonous action upon green plants, it was desirable to compare guanidine and biuret in this respect. The following solution was prepared,

Water	1000 c.c
Calcium sulphate	1 gr
Magnesium sulphate	0.5 gr
Monopotassium phosphate	1 gr
Ferous sulphate	0.1 gr

This solution was divided into 4 parts, (*a*) received guanidine hydrochlorid 0.5gr, (*b*) ammonium chlorid in equivalent quantity (0.28gr), (*c*) sodium nitrate in equivalent quantity (0.44gr), (*d*) biuret in equivalent quantity (0.464gr). Young barley plants 12. c.m. high were placed in these solutions, but after 3 days already a very poisonous action of biuret and guanidine was observed, the plants having withered almost completely, while the control plants *b* and *c* were perfectly healthy.

The amount of guanidine and of biuret were now reduced to one tenth of the former quantity and plants of the same size placed into these solutions on November 26th.

On Dec. 11. the guanidine plant was dead, but not yet the biuret plant. This however did not show any further development, the larger leaves all bleached and died off one after the other and on January 20 only the youngest leaf was still green.

[1] The urea even in the high dilution of 0.5 per mille injured the plants after a time. It is probable that the urea is readily split up into ammonia and carbonic acid in the cells and the nascent ammonia killed the chlorophyll bodies. Cf. Bul. of the College of Agr., Tokyo, V l, IV, 413.

Conclusions.

1. Chlorophyll bearing plants are injured by guanidine even in a ditution of 0.1 per mille. Biuret is somewhat less poisonous.

2. Fungi cannot utilize guanidine as a source of carbon, but only as a source of nitrogen.

Physiological Observations on Bacillus Methylicus.

BY

T. Katayama.

Some time ago[1] the writer has communicated a note on the general occurence of *Bacillus methylicus* in various soils of Japan. He has however paid also attention to the physiological properties of this microbe which will be treated upon in the following lines. It was already mentioned that a perfectly colorless variety of the reddish *Bac. methylicus* occurs frequently in soils, which behaves essentially like that of a weak reddish color. Since I had observed that in the nourishing solution with sodium formate as exclusive organic material besides the *Bac. methylicus* also some other microbes[2] frequently developed, although only in minute quantities, when the solutions had been inoculated from soils, special care in the preparation of pure cultures was necessary and I repeated therefore several times cultures in formate solutions from the colonies obtained on gelatin plates. Thus pure cultures were obtained which served for the following tests.

As regards my observations on the appearance of the colonies they agree essentially with those of *Loew*, nevertheless they may be especially mentioned.

The cells grown in the formate solution are 0.8–1 μ. thick and 2–2,5 μ. long, but they are smaller and shorter when cultured on agar or gelatin plate for 1 or 2 days, being only 0.5–0.8 μ. thick and 1–1.5 μ. long. It has no motion and is not colored after *Gram*. The formate

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

[2] Also traces of a red yeast and a mycelium fungus were noticed sometimes.

culture solution becomes gradually alkaline, and required after 5 weeks 2.8 c.c of a 1.5% sulphuric acid for neutralization for 100 c.c.

Bouillon: becomes turbid after 24 hours (20°C.), and a film is formed after a few weeks which sinks to the bottom on shaking. No growth takes place anaerobically (after *Buchner's* method) in bouillon, not even on addition of sodium formate.

Sugar bouillon: Development more rapid, ring on the surface, sediment after 3 days but no gas.

On gelatin plate: White round colonies, but when the gelatin is gradually liquefied the margin of the colony becomes irregular and radiated lines from the center are formed.

On agar plate: Colony is round, milky white and somewhat elevate.

Agar streak: White featherlike colony along the streak, porcelain-like luster, margin irregular.

Agar stab culture: White colony and on the surface only.

On potato: White thin stratum in the begining of this culture, later on a little elevated.

In sodium formate solution at 20°C: Turbidity, gradual formation of films of very weak reddish color or white.

In milk: The rim on the surface is colored light yellowish, no coagulation after two weeks, the reaction not at all acid, in contrary weak alkaline, odor very weak rancid, but not putrid.

Indol reaction is not obtained from old bouillon culture.

Against higher temperature, our microbe has but little resistance power. In a bouillon culture, it is killed after 5 minutes at 60°C, while it remains still alive at 50°C.

Behavior to Nitrate. In a culture solution containing 0.3% sodium acetate as the only organic material, further 0.2% KNO_3, 0.2% K_2HPO_4, 0.02% $MgSO_4$, *Bac. methylicus* develops very well in the form of white films and flocculi. This solution gave after 3 weeks a decided reaction for nitrous acid after *Gries*. Ammonia was not formed by the reduction of nitrate.

In a similar solution in which the nitrogen was applied as potassium nitrite (0.1%), the growth was much weaker than in nitrate; no gas was developed.

Behavior to atmospheric nitrogen. A culture solution, 100 cc, containing 0.3% sodium acetate, 0.2% K_2HPO_4 and 0.02% $MgSO_4$, contained in a large *Erlenmyer's* flask of 500 c.c capacity was inoculated with *Bac. methylicus* and kept in the incubator at 20°C. Slight opalesence becoming a little stronger after several weeks was noticeable. No farther development took place.

Behavior to urea. Culture solutions containing as sole organic matter 0.5% urea showed when infected with *Bac. Methylicus* after several weeks a slight turbidity, and faint reactions for ammonia, while the control flasks without infection remained unchanged. I hope to decide soon whether a faint impurity in the urea has enabled the minute bacterial growth.

Behavior to pepton. A culture solution containing 0.5% pepton, 0.2% K_2HPO_4 and 0.02% $MgSO_4$ developed a good growth of *Bac. methylicus*, but there was no trace of putrefaction noticeable. The reaction remained almost neutral and the biuret reaction was still obtanied after 3 weeks. There was some ammonia produced.

Behavior to ammonium humate. With regard to the presence of humic acid and of *Bac. methylicus* in the soil it seemed of special interest to observe the behavior of this microbe towards humus. A culture solution containing 0.2% ammonium humate as the only organic food showed after two weeks a thick film and a bacterial sediment. In the control solution containing besides ammonium humate 0.3% sodium acetate the growth was, however, much more luxuriant.

Behavior to starch, cane sugar, and glycose. In culture solution in which the only organic matter was starch in form of a 0.2% starch paste, no hydrolysis of starch was noticed; bacterial growth was rather insignificant.

In neutral culture solution containing 0.5% cane sugar as the only organic material growth took place but no inversion of cane sugar was noticed after several weeks.

In culture solution containing 0.5% glycose a good growth was noticed,

but the production of acidity by oxidation was only very minute, 100cc. requiring after two weeks only 1.8 c.c of a 1% baryta water.

Behavior to mannitol. In a culture solution containing 0.5% mannitol, a trace of acidity but no reducing sugar was observed; the acid is produced probably by oxidation; 100 c.c required after 3 weeks only 3.1 c.c of a 1% baryta water. The growth was good but not so luxuriant as with glucose.

Summary.

Bacillus methylicus cannot utilize the free nitrogen of the air.

Bac. methylicus forms no enzyms which can hydrolyse starch, cane sugar or proteins; it can not produce either phenomena of putrefaction or of fermentation. It cannot grow in absence of air.

Bac. methylicus can utilize humic acid as food.

Difference between Bac. methylicus and Bacterium formicicum.

After this investigation was finished an article of *W. Omelianski* appeared on the decomposition of formic acid by microbes.[1] He isolated a bacillus from horse excrements which was able to decompose anaerobically formates with development of hydrogen and carbonic acid, but the presence of pepton or bouillon was necessary.

He believes that his bacillus which he called *Bacterium formicicum* has some resemblance to *Bacillus methylicus* of Loew but states that his microbe cannot subsist upon formates or methylalcohol as food. Several farther essential differences will be seen from the following table:

[1] Cent. f. Bakt. XI page 177.

	Bac. methylicus Loew	*Bacterium formicum* Omeliansky [1]
Pepton or Bouillon + Sodium formate	Anaerobic: no growth at all.	Anaerobic: growth and development of gases.
	Aerobic: growth but no gas development.	Aerobic: growth with development of gas.
	No putrid odor.	Putrid odor.
Milk	No coagulation after 10 days.	Cogulation after one day.
On potato	White colonies, potato itself also not colored.	Yellowish brown colonies, potato becomes brown.
Gelatine	Slowly liquified.	Not liquified.
	Not motile.	Motile.
	Absolute aerobic.	Facultatively anaerobic.

[1] From this authors own notes.

On the General Occurrence of Bacillus Methylicus II.

BY

T. Katayama.

Former observations of *Loew* had demonstrated the occurrence of Bac. methylicus in the air of Europe and of Japan. The writer[1] had then proved its general occurrence in the surface soil from different parts of Japan.

It seemed to be of some interest to observe further to what depth it occurs in soils, and whether it is found also in the water of rivers and the ocean.

I procured in November by means of a boring stick samples of soils from various depth from a mulberry plantation, from a bare field and a forest. The surface consisted of loamy humus soil, the subsoil was clayey. The procedure was the same as described in my first communication: Infection in formate culture solution, preparing then the gelatin plate and from there colonies on potato and agar; further microscopical comparisons. The inoculation into the formate solution gave the following result after 2 weeks:

Depth.	Soil of Mulberry plantation.	Bare field.	Forest.
25 cm.	Soon a dense film	Dense film	Dense film
55 cm.	" " " "	" "	" "
65 cm.	Slow development	Thin film	Thin film
85 cm.	No growth	No growth	No growth
95 cm.	" "	" "	" "

[1] These Bulletins, V, No. 2.

The general occurrence of this microbe in the dust of the air made it very probable that it also occurs in all putrid liquids which are in contact with air, although this was doubted by *Omelianski*. The writer has infected sterilized formate culture solution repeatedly from putrid farmyard manure as well as from peptone solution that had become putrid on exposure to air and has thus obtained a growth of *Bac. methylicus*. The doubts of Omelianski are therefore not justified.

In order to decide whether this microbe occurs also in rivers the writer has collected in sterilized flasks water from the Sumida river near Akabane, above Tokyo from 10, 30 and 60 cm. depth and added to this water 0.5% sodium formate, 0.2% dipotassium phosphate and diammoniumphosphate and 0.02% magnesium sulphate, previously sterilized. After two weeks at 24°C. a thick film of Bac. methylicus had developed in the three flasks.

In order to test for the presence of Bac. methylicus in the water of the ocean, the writer has collected water near Yokosuka aboul 1½ miles from the coast and from a depth of 30 cm. in a sterilized flask of one litre capacity. There are no rivers anywhere in the vicinity emptying into the ocean. To this litre water were added the sterilzed nutrients just mentioned. After a few days at 24°C. a turbidity and a white film along the rim of the surface was developing. From this film inoculation in my former sodium formate culture solution was made and from there the characteristic colonies on potato and agar were produced. All these tests as well as the microscopic examination proved the identity of the bacillus in question with the colorless variety of the Bacillus methylicus. A further control test was made in order to observe whether the Bac. methylicus isolated from soil would also grow in the formate culture solution in presence of 3% Na Cl. Indeed a good growth was soon obtained. Hence Bacillus methylicus can not only ultilize the most various organic compounds from the peptone and sugars down to methylalcohol and formic acid, but also this lowest of the fatty acids under rather unfavorable conditions.

Various investigations on the bacteria in the oceans have been made by *Russel* in 1891, by *Fischer* in 1894 and by *Gran* in 1902. *Russel* found at a depth of 50 meters in the gulf of Naples in 1 cc. 121 bacteria, at 500 meters depth 22, while in the mud of that gulf at that depth 12500. Accord-

ing to *Fischer* most of the species found differ from those on land and are motile; he observed still many bacteria in the water from a depth of 1100 meter. *Gran* observed a certain species that can liquify agar which is of special interest, as galactan is a constituent of many marine algae and agar itself is a galactan obtained from this source. All those authors have overlooked the occurrence of Bacillus methylicus in sea water.

Conclusion.

Bacillus methylicus belongs to the widest spread microbes. It occurs not only in the dust of the air, in the soils to a depth of 65 cm. and in rivers, but also in the water of the ocean. Being obligate aerobic it fulfills doubtless an important function in oxidizing organic matter wherever it occurs, in the soil as well as in the waters.

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