Microbiology. — "*Action of hydrogenions, boric acid, copper, manganese, zinc and rubidium on the metabolism of Aspergillus niger*". By Mr. H. J. WATERMAN. (Communicated by Prof. M. W. BEIJERINCK).

(Communicated in the meeting of Oct. 26, 1912).

RAULIN's object when examining the culture conditions of *Aspergillus niger* [1]) was to obtain the greatest possible weight of mould. The experimenters who after him occupied themselves with this question, likewise only considered the dry weight.

Such an investigation must needs be partial as the process of the metabolism is only roughly determined by the weight of mould. For a good insight into this process it must be observed that for instance the spore formation produces differences in the chemical composition of the obtained mould materials.

Hence, the changes of the plastic aequivalent or of the assimilation quotient should be determined many times in the course of the development; first of all of the carbon then of the other elements.

In an earlier paper [2]) I proved that changes of temperature and concentration do not modify the metabolism of the carbon and that only the velocity of this process is subject to modification.

At present I have studied the influence of various chemical compounds.

1. *Action of different rates of hydrogenions.*

The results of the referring experiments are found in Table I.

We see from it in connection with the incorrectness of these observations, caused by the small quantity of mould, that the plastic aequivalent of the carbon, in spite of the slackening of the growth and sporeforming, caused by the hydrogenions, does not undergo a convincing change.

2. *Action of different boric acid concentrations.* [3])

Analogous results as for the hydrogenions were found with boric acid as seen in Table II.

In lower concentrations of about 0,06% the plastic aequivalent remains almost unchanged.

The slight lowering observed at higher concentrations may be

[1]) J. RAULIN, Etudes chimiques sur la végétation, Paris 1870.

[2]) H. J. WATERMAN, Beitrag zur Kenntnis der Kohlenstoffnahrung von *Aspergillus niger*, Folia Microbiologica, Holländische Beiträge zur gesammten Mikrobiologie 1912 Bd. I p. 422.

[3]) Also compare: J. BÖESEKEN and H. J. WATERMAN, Folia Microbiologica I (1912) p. 342.

TABLE I.

Culture liquid: 0,15 gr. paraoxybenzoic acid p. 50 cm³ tapwater. Temp. 32—33° C. Anorgani food: 0,05% NH_4Cl, 0,05 KH_2PO_4, and 0,02% $MgSO_4$.

No.	H-ions in grams p.L. given as sulf acid.	Expressed in c.m³. N. H_2SO_4 p. 100 cm³ of the culture liquid	Course of development[1]) after 2	5	9	27,28	40	Plastic. Aeq. of carbon after 27—28	90 day
1	0	0	++	++++	++++++ many spores	as after 9 days		57%	
2	0	0	++	++++	= 1			47 „	
3	$0,4 . 10^{-5}$	0,19	++	++++	= 2		As after 9 days; the quantity of spores diminishes considerably from No. 5 to No. 9.		55%
4	$1,1 . 10^{-5}$	0.58	++	++++	= 3				49 „
5	$1,8 . 10^{-5}$	0.97	++	++++	+++++ [2])				48 „
6	$2,1 . 10^{-5}$	1,17	++	+++	+++ ++ few spores				44 „
7	$2,8 . 10^{-5}$	1,56	+	++	+++ [2]) few spores				43 „
8	$3,3 . 10^{-5}$	1,96	+	++	+++ [2]) few spores				46 „
9	$3,9 . 10^{-5}$	2,35	–	+	+++ [2]) few spores				43 „
10	$4,4 . 10^{-5}$	2,74	–	–	?				
11	$5,7 . 10^{-5}$	3 92	–	–	–				

TABLE II.

50 cm³ tapwater, in which dissolved 1 gr. glucose, 0,15% NH_4NO_3, 0,15% KH_2PO_4, 0,06% $MgSO_4$. Temp.: 33 34° C.

Nr.	Weight of boric acid added in %	Development after 2	6	Plastic aequiv. of the carbon after 7 days
1	0	+++	vig. growth, many spores	36 %
2	0,01	+++	„ „ „ „ „	36,5 „
3	0,02	+++	„ „ „ „ „	35 „
4	0,06	+++	„ „ „ „ „	34 „
5	0,2	+++	„ „ „ „ „	31 „
6	0,5	++	+++++, few spores	30 „
7	1,0	+	+++, no spores	—

[1]) The differences in vigour of mycelial growth are indicated by „+", „++", „+++" etc.
[2]) The mycelium is yellow.

explained by the formerly described mutation [1]) occurring under the influence of boric acid.

From these observations it follows that the metabolism of the carbon, in opposition to the velocity of the growth and spore production, changes little by the said chemical influences.

3. *Action of copper.*

Whilst RAULIN [2]), proved that coppersulfate in strong concentrations is noxious to the development of *Aspergillus niger*, RICHTER and ONO put the question whether copper in very dilute solutions may act favourably.

ANDREAS RICHTER [3]), who stated that in absence of zinc even addition of $\frac{1}{150000000}$ gr.mol. coppersulfate per L. caused the weight of mould to decrease, answers this question negatively.

N. ONO [4]) came to an opposite result.

The observations of ONO and RICHTER need not, however, be in contradiction with each other as it is not certain that they cultivated under the same circumstances, although ONO endeavoured to do so.

ONO's experiments especially are deficient in as much as the velocity and the nature of the metabolism are not sufficiently separated. For this reason I have once more made an analogous investigation.

The chemicals used were of KAHLBAUM's and of great purity.

The distilled water was once more purified by redistillation in an apparatus of Jena glass joined by a glass tube to a tin cooler [5]), and then kept for use in Jena flasks. The cultivation took place in ERLENMEYER flasks of Jena glass of 200 cm^3. capacity.

The composition of the culture liquid was:

0.15 % ammoniumnitrate
0.1 „ potassium-chloride
0.1 „ magnesiumsulfate (crystallised)
0.05 „ calciumnitrate (free from water)
0.05 „ fosforic acid (crystallised)
2 „ glucose.

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[1]) H. J. WATERMAN, These proceedings, June 1912. Vol. XV, p. 124.

[2]) l. c. p. 136.

[3]) A. RICHTER, Centralbl. f. Bakteriol. 2e Abth. Bd. 7 (1901) p. 417.

[4]) N. ONO. Centralbl. f. Bakteriol. 2e Abth. Bd. 9 (1902) p. 154.

[5]) Corks and such like material were avoided.

Each culture tube was filled with 50 cm³. of the above liquid and coppersulfate was added in different concentrations.

After boiling spores of *Aspergillus niger* (Form I) [1]) were inoculated.

The observed development is described in Table III; the formation of but few spores is caused by the use of the said chemicals free from manganese, as is further explained in Table V.

TABLE III.

Temp. 34—35° C.

Nr.	Coppersulfate added ($CuSO_4 . 5 Aq.$)		Course of development after			
	In milligr.	In Gr. mol. p. Litre	1	3	5	9 days
1	Control	—	+	rather vigorous, hardly any spores	vigorous, hardly any spores	vigorous, only few spores
2	0,01	$\frac{8}{10\,000\,000}$	+	= 1	= 1	vigorous, very few spores
3	0,1	$\frac{8}{1\,000\,000}$	+	= 2	= 2	vigorous, hardly any spores
4	1,0	$\frac{8}{100\,000}$	+	++	+++, hardly any spores	++++, hardly any spores
5	3,5	$\frac{2,8}{10\,000}$	+	++	++, hardly any spores	+++, hardly any spores
6	9	$\frac{7,2}{10\,000}$	+	++	+++, hardly any spores	+++, hardly any spores
7	26,5	$\frac{2,12}{1000}$	+	+−++	++, hardly any spores	+++, hardly any spores
8	52	$\frac{4,16}{1000}$	+	+	++, few spores	++++, hardly any spores
9	100	$\frac{8}{1000}$	+	+	++, few spores	+++, hardly any spores
10	252	$\frac{2}{100}$	?	—	?	+, hardly any spores
11	505,5	$\frac{4}{100}$	—	—	after two months' growth, but probably with mutation	
12	1000	$\frac{8}{100}$	—	—	—	—

[1]) Compare H. J. WATERMAN. These Proceedings June 1912.

We see that already $\frac{8}{10000000}$ gr. mol. coppersulfate strongly diminishes the production of spores.

The velocity of the mycelium formation as well as the assimilation of glucose are also slackened by the coppersulfate (Comp. Nr. 4 and the following Nrs with Nrs 1—3).

By determining the quantity of dry substance [1]) and the carbonic acid obtained from it by combustion on one hand, and on the other, by determining the polarisation [2]) and the reduction number by titration after FEHLING, by which the assimilated glucose could be computed, the plastic aequivalent of the carbon could be fixed.

In Table IV the results of these experiments are united.

TABLE IV [3]).

Metabolism of *Aspergillus niger* under influence of different coppersulfate concentrations. Nine days after inoculation.

Nr.	Obtained dry weight in milligrs.	Mgr. CO_2 at combustion of the mould material	Assimilated glucose in %	Plastic aeq. of the carbon.	
1	306	530	100	36,0%	
2	318,5	556	100	38,0 „	
3	325,5	563,5	100	38,5 „	
4	377	643,5	100	44,0 „	
5	148	257,5	52	34 „	
6	190,5	331	57	39,5 „	±
7	83	146	31	32 „	
8	112	192,5	32	41 „	
9	89,5	158.5	26	41,5 „	
10	6,5	—	6	—	
11	—	—	0	—	
12	—	—	0	—	

[1]) Dried at 105° to constant weight.

[2]) Determined by the saccharimeter of SCHMIDT and HAENSCH. It is proved that in the Nrs. 1—6 a small quantity of a hitherto unknown polarisating substance occurred, not reduced by "FEHLING".

[3]) Compare Table III.

We thus observe that the addition of $\frac{8}{100000}$ gr. mol. coppersulfate considerably enhances the weight of mould after 9 days; it is more than 70 milligr. greater than that obtained without addition of copper.

Already before [1]) I explained that the non-formation of spores commonly coincides with the accumulation of glycogen in the mould and with a high plastic aequivalent of the carbon.

This we find confirmed here, compare for instance Nrs. 1, 2, 3. 4.

The values of the then following numbers are not very exact. That they are notwithstanding mentioned is to make clear that even considerable copper concentrations (N°. 9) do not change the character of the metabolism. The decrease in quickness of the assimilation of glucose is very obviously caused even by slight quantities of copper.

Whether the greater mould production may be called favourable is doubtful, the sporeforming being retarded.

4. *Action of manganese.*

BERTRAND and JAVILLIER [2]) found that addition of manganese enhances the weight of mould whilst it was also stated that this element is fixed in the organism. It also proved necessary for the spore forming [3]).

By a very minute examination BERTRAND succeeded in showing that even addition of $\frac{1}{10000000000}$ manganese made the weight of mould rise considerably. As will be seen from Table V the addition of manganese had especially brought about changes in the velocity of the glucose assimilation. For the rest, my experiments with manganese have confirmed those of BERTRAND and JAVILLIER.

The composition of the nutrient liquid was:

very pure distilled water in which dissolved:

0.15 % ammoniumnitrate
0.1 „ potassium chloride
0.1 „ magnesiumsulfate (crystallised)
0.05 „ calciumnitrate (free from water)
0.05 „ ammoniumfosfate
0.05 „ fosforic acid (crystallised)
2.— „ glucose

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[1]) H. J. WATERMAN, Folia Microbiologica I. (1912) p. 422.

[2]) BERTRAND et JAVILLIER. Influence du manganèse sur le développement de l'Aspergillus niger. C. r. **152** (1911) p. 225; Ann. de l'Institut Pasteur T. **26** (1912) 25 Avril p. 211.

[3]) BERTRAND. Extraordinaire sensibilité de l'Aspergillus niger vis à vis du manganèse. C. r. **154** (1912) p. 616.

TABLE V.

Temp. 34—35° C.

Influence of manganese on the spore-formation and metabolism of Aspergillus niger.

Nr.	Added $MnCl_2 . 4$ Aq.		Course of development after				Dry weight obtained after		Milligr. CO_2 at combustion of the mould material after 35 days	Assimilated glucose in pCt. after		Plastic Aequivalent of the carbon after 35 days
	Milligr.	Grammol. p. L.	2	3	4	5 days	4	35 days		4	35 days	
1	0	0	+++++	vigorous, hardly any spores	vigorous, very few spores	few spores	412	265	438	nearly 100 pCt.		30 pCt.
2	0,0001	$\frac{1}{100\,000\,000}$	+++++	vigorous, hardly any spores	vigorous few spores	rather many spores	undetermined	undetermined	undetermined	undetermined		
3	0,001	$\frac{1}{10\,000\,000}$	+++++	vigorous, beginning spore-form.	vigorous, rather many spores	many spores	410	„	„	100 pCt.		
4	0,01	$\frac{1}{1\,000\,000}$	+++++	idem	idem	„ „	424	„	„	„		
5	0,1	$\frac{1}{100\,000}$	+++++	idem	idem	„ „	418	„	„	nearly 100 pCt.		
6	1	$\frac{1}{10\,000}$	+++++	idem	idem	„ „	undetermined					
7	5	$\frac{0,5}{1000}$	+++++	idem	idem	„ „	„	292	477	undetermined	100 pCt.	32,5
8	10	$\frac{1}{1000}$	+++++	idem	idem	„ „	„	294	485	„		33
9	25	$\frac{2,5}{1000}$	+++++	idem	idem	„ „	„	290	484	„		33
10	50	$\frac{5}{1000}$	+++++	idem	idem	„ „	„	undetermined	undetermined	„		
11	100	$\frac{1}{100}$	+++++	idem	idem	„ „	„	302	510	„		35
12	250	$\frac{2,5}{100}$	+++++	idem	idem	„ „	„	306	—*	„		
13	500	$\frac{5}{100}$	+++++	idem	idem	„ „	„	321	—*	„		
14	1000	$\frac{1}{10}$	++++	idem	idem	„ „	„	318	—*	„		

Into each ERLENMEIJER flask of Jena glass (200 cm³ capacity) 50 cm³ of the above liquid was introduced and manganese in different concentrations was added.

For the result see Table V.

Quite as in the preceding experiment every nr. consisted of several flasks. Taking this into consideration, the extreme sensibility of *Aspergillus niger* as to manganese, already observed by BERTRAND, was with certainty confirmed. Without manganese hardly any spores are formed after four days.

In spite of the observed favourable influence of manganese on the production of spores no important modifications in the metabolism of the carbon occur (Table V).

We may thus conclude that the numbers given by BERTRAND [1]) for the dry weight with and without addition of manganese relate only to the velocity of the metabolism.

Is it necessary or desirable to distinguish elements such as manganese from others as carbon, nitrogen, etc. which occur in the organism in great percentages? Have we to reckon manganese among the purely catalytic elements, in opposition to carbon as a plastic one? In my opinion there is no sufficient reason for such a marked separation. The only important difference is that elements as manganese form an extremely small permanent percentage of the organism. It is, however, very well possible that this difference is only apparent. The circulation of manganese may for instance be much quicker than that of carbon, so that the concentration in one special cell may for a time have been relatively high. It is not, however, possible to detect this by analysis of the whole mould layer.

5. *Action of zinc.*

Since RAULIN had already supposed that zinc acts favourably on the weight of mould, JAVILLIER [2]) showed with certainty that small quantities of zinc considerably increase this weight. At the same time he proved that zinc is fixed in the mycelium [3]). Moreover, BERTRAND and JAVILLIER [4]) studied the joint action of zinc and man-

[1]) BERTRAND C. r. **154** (1912) p. 616.

[2]) JAVILLIER, Sur l'influence favorable de petites doses de zinc sur la végétation de *l'Aspergillus niger*, C.r. **145** (1907) p. 1212.

Also compare BERTRAND et JAVILLIER, Sur une methode permettant de doser de très petites quantités de zinc C. r. **143** (1906) p. 900; **145** (1907) p. 924.

[3]) Sur la fixation du zinc par *l'Aspergillus niger*. C. r. **146** (1908) p. 365.

[4]) BERTRAND et JAVILLIER, C. r. **152** (1911) p. 900; C. r. **153** (1911) p. 1337. Cf. also Ann. de l'Inst. Pasteur T. XXVI (25 Juillet 1912) p 515.

ganese, which proved more favourable than that of each of these elements separately.

In his last communication JAVILLIER [1]) mentioned that the constant relation between the assimilation of sugar and the production of mould, which is nearly 3 : 1, sometimes became 8 : 1 by leaving out zinc, that is to say, addition of zinc should allow the organism to use less food; besides, the assimilation of nitrogen and of the other anorganic elements changed according as zinc was added or not.

Hitherto I have not been able to confirm JAVILLIER's results. Addition of zinc caused but little change in the metabolism of the carbon, but again the velocity of the glucose assimilation was modified.

Addition of stronger zinc concentration: $\frac{75}{100000}$ gr. mol. $ZnCl_2$ p. L. caused a distinct, albeit slight increase of the plastic aequivalent of the carbon, but it was accompanied by non-formation of spores.

In many respects, thus, the action of zinc resembles that of copper. As with this element the addition of slight quantities of zinc, which exerts no perceptible influence on the production of spores, causes hardly any change in the weight of mould.

So, nutrient solutions containing $\frac{7}{100.000.000}$, $\frac{7}{10.000.000}$ and $\frac{7}{1.000.000}$ gr. mol. $ZnSO_4$ 7 Aq. p. L. produced after four days respectively 407, 410 and 417 milligrs. of dry material, whilst analogous experiments, without addition of zinc, produced 406 and 408 milligrs.

The fact that stronger concentrations of zinc check the forming of spores (see above) which had also been observed by SAUTON [2]) and JAVILLIER [3]), BERTRAND [4]) tries to explain by the relation existing between the quantity of manganese present on one side, and the produced mould on the other.

Thus BERTRAND says: "Lorsque au milieu nutritif on n'ajoute ni fer, ni zinc, ou seulement du fer ou du zinc, les mycéliums qui prennent naissance sont si réduits que le rapport du manganèse, introduit volontairement on non, au poids de matière organique formée, peut

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[1]) JAVILLIER, Influence du zinc sur la consommation par *l'Aspergillus niger*. de ses aliments hydrocarbonés, azotés et minéraux, C. r. **155** (1912) p. 190.

[2]) B. SAUTON, C. r. **151** (1911) p. 241.

[3]) M. JAVILLIER et B. SAUTON, Cr. **153** (1911) p. 1177.

[4]) G. BERTRAND, C. r. **154** (1912) p. 381.

être suffisant à la formation des conidies." On the contrary the greater the proportion of the quantity of mould material with respect to the manganese present, the smaller the production of spores.

This explanation is not, however, in accordance with my observations as the produced quantity of mould was only very little increased by the addition of zinc.

But like BERTRAND I have observed that by adding manganese, in spite of the presence of zinc, the production of spores is furthered.

Notwithstanding BERTRAND's excellent investigation only few of the factors are known which determine the formation of spores.

It is proved, however, that in the hitherto treated cases slackening of the spore formation is combined with a great plastic aequivalent of the carbon.

6. *Substitution of rubidium to potassium.*

In 1879 NÄGELI [1]) made some experiments with rubidium and caesium [2]) on the metabolism of *Aspergillus niger* from which he concluded that these elements could replace potassium.

BENECKE [3]), who studied this question more in detail, proved that by replacing potassium by rubidium the production of mycelium was normal, but that sporeformation was inhibited.

He found that the dry weights of the rubidium moulds at the lower Rb.concentrations were somewhat higher, in other cases again lower than those obtained in a medium containing potassium. In stronger concentrations rubidium retarded the growth and only insignificant coats of mould appeared which did produce spores, which fact BENECKE could not account for. Probably the presence of potassium, if large quantities of rubidium salt are used, then becomes of importance in relation to the small weight of mycelium.

The results obtained by NÄGELI and BENECKE are here chiefly confirmed as appears from what follows.

If instead of potassiumchloride rubidiumchlorid is used the formation of mycelium remains the same. The "rubidium moulds", however, are distinguished from those cultivated with potassium by their being

[1]) C. v. NÄGELI, Sitzungsberichte d. math. phys. Classe d. k. b. Akad. d. Wiss. zu München vom 5 Juli 1879.

[2]) I have proved that caesium cannot replace potassium.

[3]) W. BENECKE, Ein Beitrag zur mineralischen Nahrung der Pflanzen, Ber. d. deutschen botan. Gesellschaft 1894 S. 105.

Die zur Ernährung der Schimmelpilze notwendigen Metalle, Jahrbücher für wissenschaftliche Botanik Bd. **28** (1895) S. 487.

covered with only a small quantity of spores ; the rubidium mycelium is moreover more intensely yellow than in normal cases, when it often is nearly colourless.

The presence of rubidium in the said concentrations when kalium (0,1 % KCl) is present has no influence on the spore formation and on the yellow-colouring of the mycelium. (See Table VI). Here it may be added that also the addition of 0.05 % manganesechloride accelerates the spore production.

For the experiment I prepared two culture media of the following composition.

Medium A:

Distilled water in which dissolved
0.2 % ammoniumfosfate
0.1 ,, potassiumchloride
0.07 ,, magnesiumsulfate
0.035 ,, calciumchloride
2 ,, glucose
Some drops of a dilute fosforic acid solution.

Medium B:

Composed like A.; only instead of 0.1 % KCl, 0.1 % RbCl was added.

In the careful investigation of BENECKE there is wanting an exposition of the relation between the assimilated food and the weight of mould in connection with time.

The results of more exact experiments are united in Table VII.

TABLE VI.

Temp. 33° C.

50 cm³. of the above solutions were introduced into 200 cm³. Erlenmeyer-flasks of Jenaglas and after boiling inoculated with *Aspergillus niger*.

Nr.	Composition of the culture liquid	Growth and spore forming after		
		2	4	9 days
1 [1])	50 cm³ of A	++++, hardly any spores	very vigorous rather many spores	very vigorous, many spores, mycelium light yellow
2	50 cm³ of A + + 0,1% KCl	idem	idem	idem
3 [2])	50 cm³ of B	idem	very vigorous, very few spores	very vigorous, beginning of spore form. mycel. orange-col. [3])

[1]) In triplo
[2]) In duplo.
[3]) The beginning of spore formation (Nr. 3) is probably caused by the presence of but slight quantities of potassium.

TABLE VII.

Temp. 34–35° C.

Very pure distilled water in which dissolved: 0,15% ammoniumnitrate, 0,05% fosforic acid (crystallised), 0,1% magnesiumsulfate (crystallised), 0,1% calciumnitrate (free from water), 2% glucose (free from water). 50 cm³ of the above solution was introduced into carefully cleaned ERLENMEIJER flasks of Jena glass, then added either 0,1% KCl, or 0,1% RbCl.

Nr.	Added	Growth and spore formation						Obtained dry weight		Mgr. CO_2 at combustion of the mould material		Plastic Aeq. of the carbon	
		after 1	2	3	4	6 [1])	18 [1]) days	after 6	18	6	18	6	18 days
1	0,1 % KCl	+	++++, not yet spores	rather vigorous, hardly any spores	vigorous, hardly any spores	vigorous, beginning spores form.	vigorous, rather many spores	356		594		40,5%	
2	0,1 „ KCl	+	++++, not yet spores	rather vigorous, hardly any spores	vigorous, hardly any spores	vigorous, beginning spores form.			217		374		25,5%
3	0,1 „ RbCl	+	++++, not yet spores	rather vigorous, hardly any spores, mycelium yellow	vigorous, hardly any spores, mycelium yellow	as after 4 days		377		609,5		41,5%	
4	0,1 „ RbCl	+	++++, not yet spores	rather vigorous, hardly any spores, mycelium yellow	vigorous, hardly any spores, mycelium yellow	as after 4 days	vigorous, rather few spores		268		424		29%

[1]) All the glucose has disappeared from the solution.

From these it follows that the nature of the metabolism of the carbon does not change by substituting rubidium to potassium. The rubidium mycelium only proves to contain more glycogen as is shown by the greater plastic aequivalent of the carbon.

We likewise perceive that also without production of spores the digestion of the intermediary products is possible, for in spite of the fact that after 18 days at the rubidium experiment only few spores appear, the plastic aequivalent of the carbon is lowered from 41.5 to 29%,

Summary.

1. Addition of 2.35 cm³ normal sulfuric acid per 100 cm³ culture liquid and of 0.5 % boric acid but feebly influences the plastic aequivalent of the carbon. In the case of the boric acid we must ascribe the observed changes to mutation.

2. The action of the factors that govern the development of *Aspergillus niger* must not be partially judged; thus, a high weight of mycelium cannot always be called favourable. This is not sufficiently taken into consideration by ONO, RICHTER, BERTRAND and JAVILLIER. So it was proved for the action of certain concentrations of coppersulfate, zincchloride and zincsulfate, that these salts considerably increase the plastic aequivalent of the carbon, whereas the increase of the weight of mould is proportional to the retarded spore production.

Very dilute zinc solutions $\left(\frac{7}{100000000} - \frac{7}{1000000}\right.$ gr. mol $.ZnSO_4.$ $\left.7\ Aq\ p.\ L.\right)$ have no influence. Coppersalts counteract the spore forming in all concentrations.

3. Presence of manganese in minimal quantities does not change the plastic aequivalent of the carbon; it only acts on the velocity of the metabolism.

The quantities of dry substance found by BERTRAND should be considered as values indicating the velocity of the process.

4. By replacing potassium by rubidium the spore formation is counteracted, the weight of mould is increased, and the metabolism of the carbon (i. e. the change of the plastic aequivalent and of the respiration aequivalent in connection with time) remains unchanged.

Finally my hearty thanks to Professor Dr. M. W. BEIJERINCK and Professor Dr. J. BÖESEKEN for their assistance in this investigation.

Laboratories for Organic Chemistry and
Delft, October 1912. *Microbiology of the Technical University.*

Pathology. — *"On a micro-organism grown in two cases of uncomplicated Malignant Granuloma."* By ERNESTINE DE NEGRI and C. W. G. MIEREMET. (Communicated by Prof. C. H. H. SPRONCK).

(Communicated in the meeting of September 28, 1912).

In recent years Malignant Granuloma, also called Lymphomatosis granulomatosa or HODGKIN's disease, has occupied the attention of many writers and researchers, in consequence of which some more light has been thrown upon the subject after a long period of obscurity.

For all this, the etiological evidence brought forward in the study of this incurable disease is still extremely limited.

In 1832, it is true, HODGKIN [1]) published the history of some cases and autopsies which may, to a certain extent, bear on the disease we are about to discuss, but its etiology was not dwelt on in the literature before many years later.

No attempt whatever had been made to distinguish by differential diagnosis the various diseases, characterised by glandular swellings and enlargement of the spleen, until VIRCHOW, in 1845, described leukaemia as a well defined disease. Next, in 1865, COHNHEIM distinguished pseudoleukaemia as a disease of the lymphatic apparatus resembling leukaemia, but differing from it by the absence of the typical bloodpicture. Since COHNHEIM the term pseudoleukaemia has again and again been misapplied to a congery of glandular diseases; others again added the epithet "tubercular" to it, so that in spite of COHNHEIM's discovery, the confusion was again as great as before.

Neither did BILLROTH [2]) confine the term "malignant lymphoma", a name often given to malignant granuloma, to one special affection of the glands, as he himself says in his paper on Multiple Lymphome.

STERNBERG [3]) was the first to describe in an elaborate histological investigation a definite group of cases, thereby leading the way for later workers. He was likewise the first to discuss at length the etiology of the disease, as appears distinctly from the title of his publication: "Ueber eine eigenartige unter dem Bilde der Pseudoleukamie verlaufende Tuberkulose des Lymphatischen Apparates." However the etiology, suggested by the title, is not nearly ascer-

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[1]) 1832 HODGKIN. On some morbid appearances of the absorbent glands and spleen. (Med. chir. Transact. Vol 17).

[2]) 1871 BILLROTH. Multiple Lymphome. Erfolgreiche Behandlung mit Arsenik. (Wien. Med. Woch. N°. 44 S. 1065).

[3]) 1898 STERNBERG. Ueber eine eigenartige unter dem Bilde der Pseudoleukaemie verlaufende Tuberkulose des Lymphatischen Apparates. (Zeitschr. f. Heilk. Bd XIX S. 21).