Bio-Chemistry. — *"On the action of some carbon derivatives on the development of penicillium glaucum and their retarding action in connection with solubility in water and in oil."* By Prof. J. BÖESEKEN and Mr. H. WATERMAN. (Communicated by Prof. A. F. HOLLEMAN).

(Communicated in the meeting of January 27, 1912).

I.

In our previous communication [1]) we have shown that there exists no essential difference between the benzene derivatives investigated by us, as regards their action as retarding, as well as nutrient substances for the penicillium glaucum.

The compounds readily soluble in oil exert a retarding action even in small concentrations, whereas with substances sparingly soluble in oil, this retardation only occurred with higher concentrations, but all these substances *which dissolve fairly readily in water* give, finally, a development although with compounds like salicylic acid, benzoic acid and the toluylic acids, this takes place only with very low concentrations at which a vigorous vegetation is excluded owing to the small total quantity of organic nutriment.

In each case we may take it for granted that all these substances are capable of providing the penicillium with carbon-containing material, if we only take care (by the choice of a fitting concentration) that the organism cannot overload itself with the same.

Our continued investigation has shown that the penicillium is not very particular as to its food, but that it is capable of development in solutions of nearly all the carbon derivatives.

Even chloroform, formic acid, methyl alcohol and carbon tetrachloride, given as exclusive carbon-containing nutriment, cause growth and, therefore, can yield the material from which the penicillium constructs its undoubtedly very complicated system of organic compounds.

Only a few derivatives of the highest oxidation stage of carbon, such as carbon dioxide and urea are unsuitable.

The results are shown in Table I. For the description of the experimental process we refer to the first communication.

If we survey these experiments which have been collected in this table at random from a much greater number, we notice in the first place that by far the greater number, chosen from the most varying groups of organic compounds, can promote the growth.

[1]) These Proc. of December 30, 1911, p. 608.

TABLE 1. Development of the penic. gl. on different carbon compounds The inorganic nutriment consists of $^1/_{20}$ % of potassium phosphate, $^1/_{20}$ % NH_4Cl and $^1/_{20}$ % of magnesium sulphate; *t* if not stated otherwise = 21°—22°.
Quantity of solution = 50 cc

Compound	N°.	Quant. in mg.	Development after a given number of days				Remarks.
formic acid	1	9	2—	3 ?	4+	10+	
acetic »	2	10	2+	3+	4++	10+++	
palmitic »	3		2 ?	3+	4+	10+	The pieces do not perceptibly dissolve. 3 and 5 fairly well sol. in oil
cerotic »	4		2 ?	3+	4+	10+	» » » »
stearic »	5		2 ?	3+	4+	10+	» » » »
oxalic »	6	5.5	4+	6+		11++	Temperature of the room.
malonic »	7	5.5	4+	6++		11++	» » » »
sebacic »	8	5.5	4+	6++		11 strong	Division factor oil : water = 0.3
succinic »	9	6	4+	6++		11++	» » » } very small
glutaric »	10	4	4+	6++		11++	» » »
d tartaric »	11	51	2+	3++	5++	12++	Insoluble in oil.
formaldehyde	12	9	2—	8—			
methyl alcohol	13	3.5	2+	3+	7++		
»	14	200	2+	3+	7++		
ethyl alcohol	15	4	2+	3+	7++		
»	16	210	2+	3+	7 v. strong		
glycol	17	22	2+	8+			Development remains slight.
glycolic acid	18	10.5	2+	3+	6+		» » »
lactic »	19	21	2+	6+++		9 strong	
chloroform	20		2 ?			30+++	One dr. which does not quite diss.
»	21		2—	4+	10++		
CCl_4	22		3—	4—	10+		
C_2Cl_4	23		3—	4—	10+		» » » » » » »
CClBr : CHCl	24		3—	4—	10+		
CClH : CCl_2	25		3—	4 ?	10++		
p-phenolsulf. ac.	26	3.5	2+	3++	5+++		The sulphonic acid group also proves no hindrance; in our first investigation we had, presumably, started from an impure preparation.
»	27	55	2+	3++	5+++		
benzene	28		2 ?	4+	6+	9++	One drop. Benzene mixes with oil in all proportions.
naphtalene	29		5+	7++	9++	14++	Of these hydrocarbons were added quantities of 1,5—8 mg., which, however, did not dissolve.
anthracene	30		5 ?	8+			
phenantrene	31		5 ?	8+			
pyrene	32		—	—	—		Naphtalene is very readily soluble in oil.
α naphtoic acid	33	5	5+	7+	9++	14++	Very readily soluble in oil.
α naphtol	34	6	5—	7—	9—	14—	Division factor between 76-102. Both naphthols are perceptibly soluble in water.
β »	35	8	5—	7—	9—	14—	
cetyl alcohol	36	8	2—	4—	11+	30++	Fairly soluble in oil; perceptibly soluble in water.
olive oil [1]	37		2+	4++		14 strong	One drop.
urea	38	10	2—	4—	6—	30—	
glycerol [1]	39	42	2+	3++	} strong	7 } v. strong	
	40	261	2+	3+++		7	
	41	1073	2++	3+++	4 v. strong	7	
	42	1403	2++	3++	4 str. <39	7 str. som. 39	

[1] The research of A. ROUSSY C. R. **153**, 894 (1911) on the life of fungi on fats and fatty acids gives no pure result, as in the nutrient base tartaric acid was present which, as is well known, (and we can confirm this) forms a suitable source of carbon.
Owing to the presence of this acid, the growth on dilute glycerol solutions has escaped him; he has only noticed a harmful action of 10 to 12% of glycerol which is not to be wondered at, as we found already a slight diminution with a ± 3% solution (No. 42).

Besides carbon dioxide itself, formaldehyde, pyrene, urea and also the two naphthols cause no development.

As regards formaldehyde we are going to extend the investigation to still lower concentrations; urea is very closely allied to carbon dioxide, and the two naphthols have a particularly favourable division factor oil: water, *while they are still very perceptibly soluble in water*.

In the second place it strikes us that there are some compounds such as palmitic acid, stearic acid, benzene, naphthalene, cetyl alcohol, and olive oil, whose division factor olive oil: water is undoubtedly much larger than that of salicylic acid and benzoic acid and which, evidently, can yet promote the development of the mould.

These substances, however, are distinguished from the above strongly retarding substances (to which belong also the naphthols) by their *extremely sparing solubility in water*.

In order to demonstrate the significance of the rapid dissolving of the substances in water in connection with their nutrient or retarding action, we are obliged to consider the quality of the protoplasmic wall which those substances must traverse in order to be absorbed in the organism. Let it be presupposed that a too large concentration of any substance whatever will cause a retardation of the growth. Without further arguments as to the possible structure of the protoplasmic wall we may well take it for granted that it must not be put on a line with a layer of oil, without further evidence.

So long as it forms a part of the living organism it must be regarded as a wall limited by an aqueous liquid, and therefore as a membrane which, in a certain sense, is protected by a layer of water.

If we were dealing with an oily layer immediately accessible, all substances readily soluble in oil, and particularly those insoluble in water, would penetrate and exert their retarding action.

[On the other hand, substances insoluble in oil would be unable to penetrate and thus serve as a nutriment. This point, which may be connected with the differences existing between lecithin cholesterol mixtures and true oils, will be disregarded for the present]

This, however, is evidently incorrect, for the higher fatty acids (which are very sparingly soluble in water) naphtalene, cetyl alcohol and olive oil itself, which dissolve in oil freely or very readily, do not act at all retardative on the growth of the *penicillium* and, on the whole, form in the long run a fairly good organic nutriment.

This behaviour may be explained very readily when, as stated above, we suppose that all the substances before they arrive at the protoplasmic wall must traverse a layer of water; the substances very sparingly soluble in water can perform this only in an extremely tardy manner and the concentration of these substances in the protoplasm can, therefore, increase but very slowly. If, when the concentration in the protoplasm is but small the organism can use up these substances — and the penicillium seems capable of this in a very high degree — they may act as an organic nutriment.

The substances which are readily soluble in water but very sparingly soluble in lecithine etc. such as (presumably) succinic acid, glutaric acid etc. will, of course, be prevented by this last property from rapidly overloading the organism. If these can be readily assimilated they will form a good carbon-containing nutriment.

Not merely the great division factor lipoid: water marks a substance as a narcotic, but it must be capable of entering rapidly into the organism and, besides a rapid and ready absorption in the protoplasmic wall proper, a decided solubility in water is therefore required [1]).

From this and the previous investigation it follows that from any organic substance devoid of any particularly pronounced basic or acid properties we may predict [2]) whether it will exert a strongly retarding action on the growth of lower organisms. If so, it must be (*a*) somewhat soluble in water and (*b*) dissolve considerably more in oil than in water [2]).

II.

In order to confirm the results obtained and the conclusions drawn therefrom, by a larger number of experimental data we have extended our investigations over a large number of saturated fatty acids with normal carbon chain.

These offer the great advantage that, in this series of substances we have at disposal a material of which, while retaining analogous chemical properties, the physical characters, so far as the solubility

1) Whether colloidal solutions must be regarded here as distinct from true solutions must be decided later.

2) This prediction, of course, only relates to aqueous solutions; as soon as much fat is present, for instance in butter, the retarding substance, on account of the large division factor will accumulate in the fat and can only act in a retarding manner for so far it is still present in the water-phase. We will refer to this question later on account of its practical importance.

in water and oil is concerned, undergo from term to term a continuous modification.

The preparations, which we have used, were mostly obtained

TABLE II. Development of the *penicillium* in solutions of $^1/_{20}$ % potassium phosphate, $^1/_{20}$ % ammonium chloride and $^1/_{50}$ % magnesiumsulphate which contain the saturated fatty acids as exclusive organic nutriment.
$T = 20°-21°$; quantity of liquid = 50 c.c.

Compound	N°.	quant. in mg.	Development after a given number of days				Maxim. development	Remarks
formic acid	1	9	2—	4+	6+	10+	?	small development
	2	45	2—	4—	6 ?	10 ?		
	3	91	2—	4—	6—	10—		
acetic acid	4	10	2+	4++	6+++	fairly strong	50—99	acetic acid does not dissolve in olive oil in all proportions
	5	50	2 ?	4++	6 strong	s'rong		
	6	99	2—	4++ <N°.5	6+++	fairly strong		
prop. acid	7	9.5	2+	4+	6++	10++	±48	dissolves in olive oil in all proportions
	8	48	2—	4+	6++>7	10++++		
	9	96	2—	4—	6+	10+		
n butyr. acid	10	11	2—	4+	6+	10++	somewhat <55	»
	11	55	2—	4—	6+<11	10++		
	12	110	2—	4—	6—	10—		
valeric acid	13	8.3	3++	5++	7++	10+++	far below 90.3	»
	14	90.3	3 ?	5+	7+slight	10+		
	15	95.4	3—	5+<14	7+	10+		
	16	208.4	3—	5 7	7 ?	10 —		
(Hexoic) caproic acid	17	221.1	3—	5—	7—	10—		»
	18	1 drop	2 7	4++	6+++	9 strong		
	19	2 drops	2—	4 ?	6+	9+		
heptoic acid	20	1 drop	2—	4—	6+	9++	.	2 drops no longer dissolved entirely in water
	21	2 drops	2—	4—	6—	9—		
(Octoic) caprylicacid	22	1 drop	2+	4++	6+++	9 strong		
	23	2 drops	2—	4—	6—	9—		
nonoic acid	24	1 drop	2—	4 ?	6+	9++		»
	25	2 drops	2—	4—	6—	9 —		
(Decoic) capric acid	26	very small quantity	+	++	+++	strong		Of the other fatty acids a small quantity no longer dissolves in water. The solubility of palmitic acid and stearic acid in olive oil is still very considerable > 0,4 % (of palm. acid > 1)
lauric acid	27		+	++	+++	strong		
myristicacid	28		+	++	++	++		
palmiticacid	29		2 +	4 +	6 +	9 +		
stearic acid	30		+	+	+	+		
arach. acid	31		+	+		not observed		
cerotic acid	32		+	+	+	+		

from KAHLBAUM; of the lower fatty acids the concentration was determined by titration, whereas the higher ones, if perceptibly soluble in water, were weighed out.

In the case of the practically insoluble acids a little of the substance was added; on sterilising they did not perceptibly dissolve. Of the acids soluble in water different concentrations were investigated in order to determine, if possible, the maximum of development.

For the rest, we refer to our first communication.

When, for a moment, we exclude formic acid, we notice that the fatty acids behave exactly as was to be expected from their solubility in water and in oil.

Acetic acid, favoured by its solubility in water, can serve as an exclusive carbon-containing nutriment up to fairly high concentration.

The concentration of maximum growth is situated about as high as with *p*- and *m*-oxybenzoic acid (see previous communication).

With propionic acid, *n*-butyric acid and *n*-valeric acid which are miscible with oil in all proportions, this maximum is situated much lower.

As they dissolve sufficiently in water, they can act either as a nutriment or as a retarding agent according to the concentration.

This is still very plainly perceptible with the acids C_6 to C_9 included; in very low concentration they give an excellent development but very soon, however, the maximum is attained, so that in a very dilute but still saturated solution (<2 drops to 50 cc.) no, or but little, growth takes place.

From capric (Decoic) acid upwards nothing more is noticed of a retarding action. Notwithstanding the undoubtedly very high division factor oil : water they all cause development.

Here, the solubility in water has become so trifling that the organism can no longer absorb the fatty acid rapidly (see previous communication).

As the penicillium can use up this carbon-containing material also, it becomes assimilated before the unfavourable concentration in the organism is arrived at.

As is to be expected, the growth becomes less marked with the increase in the number of carbon atoms (see palmitic compared with lauric acid) *not* owing to a strong decrease of the solubility in the fatty portion of the organism but, in the first place, to the gradual diminution of the solubility in water, which so impedes the entering into the organism that the assimilation can only take place with slight rapidity.

As regards the formic acid, the less favourable development might be due to the hydrogen ions. This, however, is not the case with the smaller concentrations as shown from an investigation as to the retarding action of the *H*-ions (see later).

It is much more likely that the slow growth is due to the simple construction and the high state of oxidation of this acid. An assimilation in the organism is here attended with grave difficulties.

We, rather, ought to be surprised to find that the *penicillium* is capable of development in a solution of formic acid as sole organic nutrient, of forming spores, etc.

In the higher concentrations this slow assimilation must also play a rôle, because in such a case the organism, not being able to use up the formic acid quickly enough, will be overloaded and the development will cease.

We notice, indeed, that the maximum development is situated much lower than in the case of acetic acid, although the division factor oil · water will, presumably, be smaller.

Summary.

An investigation was instituted as to the influence which certain carbon derivatives, when given as exclusive organic nutriment exert on the growth, and the retardation thereof, of the *penicillium glaucum*.

Here it was shown :

1. That the development may be induced by the majority of the carbon derivatives investigated, which belong to widely different groups of compounds.

2. That the growth takes place readily on compounds fairly soluble in water but not, or but slightly, soluble in oil.

3. That the growth only takes place at very low concentrations in the case of compounds which are more readily soluble in oil than in water, whereas at somewhat more considerable concentrations it is retarded or does not take place at all.

4. That this growth is not considerably interfered with when the solubility in water is exceedingly small even though the solubility in oil be very considerable.

5. That this growth is very weak or imperceptible :

a. on compounds nearly insoluble in water,

b, on simple highly oxidised compounds such as carbon dioxide, urea and formic acid.

c. on some compounds which readily dissolve in oil and are also soluble in water to a not inconsiderable extent, such as the naphthols, carbon tetrachloride, formaldehyde (3).

We have endeavoured to explain these facts by assuming that the organism is protected by a layer of water through which it has to be reached by the nutrient as well as by the retarding substances. In the case of substances soluble in water it will depend mainly on their solubility in fat whether they will penetrate the organism rapidly and eventually overload the same.

a. If they are absolutely insoluble in water they will have neither a nutrient, nor a toxical action.

b. If they are *very* little soluble in water but fairly so in oil (cetyl alcohol, palmitic acid, naphthalene) they will have a nutrient but no toxical action.

c. If they are considerably soluble in water, but still much more so in oil, they can act as a nutrient in small concentrations only, at higher concentrations they cause retardation.

d. If they are readily soluble in water but very little so in oil, they cannot act as a toxical substance, but only as a nutrient.

Finally, we have drawn from this the conclusion that an antiseptic [1]) must have a large division factor oil : water, also a sufficient solubility in the last solvent.

Delft, January 1912. *Org. Chem. Lab. Techn. University.*

Astronomy. — "*Calculations concerning the central line of the solar eclipse of April the 17th.* 1912 *in the Netherlands*". By Mr. J. WEEDER. (Communicated by Prof. E. F. VAN DE SANDE BAKHUYZEN).

(Communicated in the meeting of January 27, 1912).

Although the central line of a solar eclipse is given in the astronomical ephemerides through many points on the surface of the earth, it may be useful for the observation of the approaching eclipse to communicate a few results which have been calculated for Holland in particular. Owing to the small width of the zone of annularity in Dutch Limburg the data given in the almanacs for this eclipse are not sufficient to predict whether or no a particular place will be situated within this zone; this is obvious as the differences between the different calculations surpass the width of the zone.

This disagreement arises principally from the differences between the geocentric places of the moon which have been adopted for the calculations; the employed values of the ellipticity of the earth have had some influence too.

1) No strong acid or strong base is meant here, but a chemically-indifferent substance.