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PROCEEDINGS OF THE MEETING

of Saturday February 23, 1901.

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The following papers were read:

Botanics. — Prof. F. A. F. C. WENT: "*On the Influence of Nutrition on the Secretion of Enzymes by Monilia sitophila (Mont.) Sacc.*".

(Read January 26, 1901).

The mould Monilia sitophila is used in the West of Java to cause decomposition in cakes of Arachis seeds; these are then eaten by the Sundanese under the name of *onchom*.

Spontaneously this mould occurs on putrefying bread and wheat-flour and has also been found in France; in Java I met with this Monilia growing spontaneously on dead leaf-sheaths of the sugar-cane in the residency of Pekalongan (where onchom cakes are

unknown). Material for investigation I received by the interference of Dr. A. G. VORDERMAN, whilst the determination was done with the kind help of Prof. C. A. J. A. OUDEMANS.

Like other Monilia species this mould possesses a branched mycelium from which arise the conidia-bearing hyphae up in the air; these are strongly ramified and are often for the greater portion of their length built up of chains of conidia, which are elliptically shaped, much varying in size (from 5 to 14 μ diam.); they separate very easily, after having for a time been united by a connecting part. In rich cultures the hyphae are often united into tree-shaped masses, whilst the walls of the culture vessels are mostly coated with fringelike, downward pending, loose conidia-bearing filaments. I found that the presence of a moist atmosphere is a condition for the appearance of conidia; hence, their formation can be almost totally suppressed by keeping the air above the cultures as dry as possible, especially when the nutrient liquid is much concentrated.

Probably the fungus has yet another form of reproduction; at least I repeatedly found characteristically wound hyphae, which gave the impression of young perithecia. I did not however succeed in bringing them to further development, however much I varied the culture conditions (neither, for instance, when suddenly introducing a strongly fed mycelium into water, which has in some cases been successfully applied by KLEBS [1]).

Pigment.

Monilia sitophila is a most striking mould by its bright orange-red colour. The pigment can be solved in absolute alcohol, ether, benzol, chloroform, etc. by which a solution is obtained of gold-yellow to brown-red with a faintly green fluorescence; after evaporation of the solvent, brown, fatty drops remain; insoluble is the pigment, inter alia in water, acetic acid and hydrochloric acid. The absorption spectrum of the pigment-solution shows a dark zone, embracing the whole of the blue and violet portion of the spectrum, from about *F*.

Under the microscope the protoplasts often give the impression of being coloured uniformly orange, but the pigment is also seen lying in drops in the protoplasm. I suppose that this is always the case, but that these drops are often so small that they cannot be distinguished separately.

[1]) Jahrb. für wiss. Botanik 33. 1899, p. 513.

The mould has the remarkable property of exclusively forming this pigment when exposed to the light. If Monilia sitophila is grown in the dark, the mycelium and the conidia remain colourless; if however such a culture is placed in diffuse day-light, a light rosy tint sets in after 2 to 3 hours, which slowly passes into orange. Exposure to the light during only 15 minutes is sufficient to bring about a faintly rosy tint after a few hours, which however does not pass into orange in this case. A still shorter exposure to light seemed to cause a slight change of colour, if the action however was shorter than 5 minutes, the mycelium remained white.

It was furthermore proved that the blue and violet rays (the same which are absorbed by the pigment) are those which exert the above influence. If the mould is grown in the light, which has passed either through a kalium-bichromate solution or through a solution of the pigment itself, the mycelium continues colourless, whilst a bright orange-yellow tint appears when the culture is lighted by rays that have passed through a solution of cuprammoniumoxide.

The signification of this pigment production for the life of the plant is not yet clear to me; perhaps it protects the enzymes, produced by the mould, against the influence of the light; it is my intention to make a further investigation of this point.

Very frequently the medium on which the mould is grown, takes a brown colour, especially in old cultures. This stands in no relation whatever to the influence of the light, but, on the contrary, depends on the chemical composition of the nutrient medium. This brown colour, namely, appears only then, when the medium contains albuminous matter, peptones, or tyrosin, so that this is probably due to a secretion of tyrosinase.

Conditions of Nutrition.

Monilia sitophila thrives very well on number of natural media, such as Arachis seeds, bread, carrots, milk, broth, infusions of plums or raisins, somewhat less on the white of eggs, potatoes or sliced apples.

The use of media of exactly known composition proves that albuminous substances and peptone can serve as sources, both of carbon and nitrogen for our mould, whence the value of suchlike substances, as nitrogen food alone, is difficult to determine. Excluding these, one of the best sources of nitrogen (glucose being given as carbon nutrient), proves to be tyrosin, further asparagin, asparagin

acid, leucin, anorganic nitrates, ammonium salts and nitrites, lastly, alanin and glycocol. Bad sources of nitrogen are urea and hippuric acid, whilst kreatin and coffeïn can still less serve as such. Many of the here mentioned organic compounds can serve as carbon food too, though mostly no vigorous mycelium is formed in this case.

As sources of carbon stand foremost, besides the already mentioned albuminous matters and peptones, the carbohydrates.

Of the substances examined, we must first of all mention raffinose, whilst also the following ones can be quite well utilised as sources of carbon: starch, dextrin, maltose and cellulose, in less degree glucose, fructose, mannose and glycogen, lastly cane-sugar, galactose, lactose, arabinose, arabin and inulin. Aromatic compounds seem unfit to serve as sources of nitrogen; on the other hand several non-carbohydrates of the fat-series may serve as such: among the alcohols in the first place glycerin, further mannite, erythrite, dulcite and in very small degree ethylalcohol; of the acids (in the form of salts) may be mentioned acetic acid, tartaric acid, lactic acid, malic acid, finally also acid-amids and amidoacids, such as asparagin, asparagin acid and glycocol. Fats are bad sources of carbon, yet the mould succeeds in getting some nourishment out of them.

Although in this short paper I will not enter into ampler details concerning the conditions of nutrition, a few points are worth special mention. The optimum temperature for the development of Monilia sitophila lies at about 30° C.; at this temperature several substances can still be used as nutrients, which at ± 15° C. are valueless as such. Hence, if the object is to grow the mould at the ordinary room temperature, this will only succeed when the conditions of nutrition are well chosen, but even then, the development goes on rather slowly.

Furthermore it should be observed that the value of a nitrogen food depends on the carbon food present, and the reverse. If for instance, maltose, glucose, lactose, cane-sugar and glycerin are offered as sources of carbon, then maltose proves to afford the most vigorous development when tyrosin, glycocol, hippuric acid, kreatin, or leucin serve as source of nitrogen, whilst cane-sugar is the best source of carbon with asparagin as source of nitrogen, and finally, when alanin is used, the development at the nutrition with glycerin is three times more vigorous than with any of the other examined substances. It appears to me that the explanation of this phenomenon should perhaps be sought in the greater or smaller facility with which the plant can form proteids from the carbon and

nitrogen food which it receives. For we know from experiments with higher plants by HANSTEEN [1]), that Lemna can form proteids from asparagin and glucose, but not from asparagin and cane-sugar; on the other hand, it can form them from cane-sugar and glycocol, but not from glucose and glycocol.

Lastly, in such experiments distinction should be made between the value of a carbon food as plastic material for the production of the constituents of the plant-body and as respiration material. It seems to me that this is most evident when comparing the result of nutrition with glycerin alone, raffinose alone, and with both combined. If as food is used 100 cm. of a liquid which contains, besides 0.5 pCt. NH_4NO_3, and the other required anorganic salts 3.27 pCt. glycerin, then after about two weeks, a crop is obtained of $\pm$ 25 mgrs. (expressed in dry matter of the mould). If instead of glycerin 0.16 pCt. raffinose is taken, the crop is under the same circumstances about 19 mgrs.; if however these two are combined, so that the nutrient liquid contains 0,16 pCt. raffinose and 3.24 pCt. glycerin then the crop is 150 mgrs. In order to get an equal crop with raffinose as the only carbon food, 2,5 pCt. of this substance must be added to the nutrient liquid, whilst with glycerin such a crop is not to be obtained. This can be explained when we admit that glycerin is not a fit material for the production of protoplasm or cell-wall (at least with NH_4NO_3 as source of nitrogen), but is, on the other hand a good respiration material.

As well on an acid as on an alkaline medium the mould can grow; to 100 cm. of nutrient liquid can be added 10 cm. of $^1/_{10}$ norm. sulphuric acid, even 25 ccm. of $^1/_{10}$ norm. caustic potash, and yet development will take place.

The mould can live anaerobiontically; as well in BUCHNER's tubes, where the oxygen is absorbed by pyrogallol and caustic soda, as in a current of hydrogen, a rather vigorous development is obtained, though less than in the air. It seems to me that the development decreases, when the last traces of free oxygen are better removed, so that in complete absence of this element the development is probably quite stopped. In an atmosphere of hydrogen, CO_2 is developed and alcohol is formed.

Decompositions caused by Monilia sitophila.

Fats as well as proteids and carbohydrates are liable to certain

[1]) Jahrb. für wiss. Botanik. 33, 1899, p. 117.

decompositions when introduced into a culture fluid in which Monilia sitophila is present.

Fats are, although very slowly, splitted up into glycerin and free fatty acids. Probably the mould uses the glycerin as food. This can be easily demonstrated by growing Monilia sitophila in a fluid which contains as carbon food butter-fat or Arachis-oil or another fat and to which is added a little litmus. The development of the mould takes place very slowly and at the same time the solution is seen to grow more and more red; in the absence of fats the mould forms no acid. This decomposition is probably caused by a secreted enzyme, a lipase. If the mould grows on milk, this becomes acid, and at the same time the casein precipitates, which in my opinion, should be attributed to the decomposition of the fats of the milk. Hence, when milk, rendered free from fat by filtration, is used as medium for our Monilia, no precipitate appears but on the contrary, the slight deposit which forms at sterilisation, is gradually solved. This is a consequence of the secretion of a proteolytic enzyme, to which I shall presently return. The dark brown colour which these liquids thereby assume, is, as mentioned above, a consequence of the presence of proteids.

Nutrient gelatin is liquefied by the mould, as well in neutral, as in alkaline or feebly acid condition, in absence and in presence of free oxygen. So it was obvious that a proteolytic, more particularly, a tryptic enzyme, is secreted. If a culture is made in a peptone solution, filtered after some time, and introduced into tubes of coagulated gelatin (with addition of an antisepticum, such as toluene or thymol), then the gelatin at the surface is slowly liquefied, this does not occur when the said liquid has first been boiled; hence it is evident that a gelatin-liquefying enzyme was secreted by the mould. The quantity of this enzyme is however very small which renders its examination troublesome. Moreover the secretion proves to depend on the nutrition of the mould; it is, e.g., found when peptone is given as food, not when glycogen and NH_4NO_3 are the nutrients. I did not, however, pay much attention to this fact as something similar is much more distinctly observed with the carbohydrates and can there be better measured.

The splitting of the proteids goes certainly further than the appearance of peptones, so it is easy to state the formation of NH_3. It is also evident from the following experiment, that peptone is decomposed by the enzyme (or enzymes) in question here: When from a peptone liquid the mould is filtered and the liquid is allowed to stand with a little toluene, the rotation to the left which is a

consequence of the presence of peptone slowly decreases. This change of rotation does not occur when the liquid has first for a short time been heated to 100° C. The decomposition products of proteids are however also found in cultures to which no trace of any proteid has been added, e g. in glycerin and KNO_3 solution. These can here have only taken origin from the protoplasm of already dead cells of the mould.

I have given much attention to the action exerted by Monilia sitophila on carbohydrates. Starch, dextrin, cane-sugar and maltose are hydrolised by the mould, lactose is not changed, although, as said above, it can serve as food. Cellulose is attacked and converted into a reducing sugar, which is however evidently soon consumed as food, so that only a feeble reduction of FEHLING is observed in culture liquids where cellulose is present as carbon food. That the cellulose is attacked is easily seen under the microscope, when the mould is grown on Arachis seeds, the cell-walls are in all directions infested by the hyphae and so the cells are disjoined. I think that in this action on cellulose and in the saccharification of the starch (wherewith compared the converting of proteids and fats is very subordinate) the chief signification of Monilia sitophila as technical mould should be sought.

Cane-sugar is hydrolised into invert-sugar, maltose into glucose; in both cases there is question of enzymes, as will be nearer explained below. The saccharification of the starch also, should be ascribed to the secretion of an enzyme (or perhaps two enzymes). This saccharification can best be observed when the mould is grown on boiled rice. The tough viscous matter is slowly liquefied; whilst at first the iodine reaction is distinctly blue, it gradually grows more reddish and finally all the starch proves to have vanished. The sugar formed is d-glucose, this follows from the extent of the rotation of the polarisation plane, compared with the reduction of FEHLING and from the formation of glucosazone with phenylhydrazine acetate. During the beginning of the hydrolysis however, the rotation proves to be much greater than corresponds with the cupric-oxide reduction, when this is rated as glucose; this is a consequence of the formation of dextrin as mid-product. If the dextrin is precipitated with alcohol then the rotation and the cupric-oxide-reducing power quite correspond with those of glucose. If the conversion products are daily determined, there is found in the beginning much dextrin and little glucose; by and by the latter increases whilst the former diminishes and at length disappears, when the glucose has reached a maximum (about 43 pCt. of the weight in rice); afterwards the glucose also

decreases, evidently it is consumed by the mould. The auxanographic method of BEIJERINCK-WIJSMAN is difficult to apply whilst moulds as these soon completely overgrow an agar-agar- or gelatin-plate. Still the conversion of starch can be observed therewith, when an agar-plate is made and Monilia allowed to develop on it. When after a few days a dilute iodine solution is poured over the plate, it remains colourless at the place where the development of the mould has begun; round about a red zone is seen which gradually passes into the blue of the further portion of the plate.

From starch of different plants, under for the rest like circumstances, do not result equal quantities of sugar. I did not minutely investigate this fact; I only refer to it as it corresponds with what has before been described by me conjointly with Mr. PRINSEN GEERLIGS about Chlamydomucor Oryzae [1]).

The carbohydrates undergo still further conversions, as Monilia sitophila produces also alcohol and besides various esters; the latter cause the cultures to spread a pleasant odour, reminding of apple-essence. If these ethereal substances are distilled off they give a distinct jodoform reaction, whilst at a fractionated distillation of this product, the chief portion of the distillate, when shaken with benzoylchloride and caustic soda, produces a substance which by its smell is known as ethyl-benzoate.

Influence of the Nutrition on the Secretion of Enzymes.

The conversions of cane-sugar, maltose, and starch are caused by enzymes, which are secreted by the cells and so are to be found in the nutrient liquid. This can easily be shown by freeing the liquid with the help of filter-paper from the mycelium and the conidia of the mould and then mixing the filtrate with a solution of cane-sugar, maltose, or soluble starch, with addition of a little toluene or thymol, to prevent the growth of micro-organisms. After some time a conversion appears to have occurred, which can be measured by the change in the rotation of the polarisation plane or by the cupric-oxide reduction test, and can be qualitatively estimated by making the osazones. For control an experiment was made at the same time with the other half of the liquid, after it had been boiled a moment; with it the conversions did not take place. The enzyme (or better the mixture of enzymes) could be

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[1]) Verhandelingen Kon. Akad. v. Wet. 2e Sectie, Dl. IV, No. 2, 1895.

precipitated with alcohol; after washing with alcohol a yellow-white powder was obtained, partly soluble in water. The solution proved to possess the properties of the original liquid, though in an attenuated degree; as is known for other cases, here also alcohol seems prejudicial to the activity of the enzymes. In pure state (albeit a mixture) they are surely not obtained in this way, because, as I hinted above, decomposition products of proteids occur in every culture liquid, and these are also partly precipitated by alcohol.

Are these enzymes secreted under all circumstances? It is known that for the glands of the intestinal canal of the higher animals, the experiments of PAVLOFF and his disciples have demonstrated, that the secretion of enzymes is indirectly influenced by the nutrition, but here the presence of the nervous system makes the phenomena extremely complex, so that the idea lay at hand to seek, whether not in plants something similar might be found in simpler form. For bacteria, FERMI [1]) had observed that a gelatin-liquefying enzyme is only produced in the presence of food containing proteids, whilst WORTMANN [2]) had thought to find a similar fact for diastase; but it should be called to mind, that the latter investigation was not done with pure cultures. BROWN and MORRIS [3]) have shown that embryos of grasses secrete no diastase when growing in strong sugar solutions. KATZ [4]) thinks that Penicillium glaucum would secrete no diastase when a sufficient quantity of cane-sugar or glucose is present in the nutrient liquid; to my opinion, however the method of investigation used does not allow to draw this conclusion. Finally DUCLAUX [5]) gives some brief remarks concerning Penicillium glaucum and a not nearer determined Aspergillus, which secrete certain enzymes only when they are fed in a special way.

Monilia sitophila enabled me more amply to study similar phenomena. As I said above, the proteolytic enzyme is secreted only with a particular nutrition, but I have not nearer investigated this point, because I wished to measure the quantity of enzyme and this can only be done exactly, when the conversion products can also be well determined. With the amylolytic enzyme we meet with the difficulty, that we do not know whether this is really a simple conversion or a co-operation of more enzymes. Hence, I wish only

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[1]) Centralblatt für Bakter. u. Parasitenk. Bd. X. 1891. p. 401.

[2]) Zeitschr. f. physiol. Chemie. Bd. VI. 1882. p. 287.

[3]) Journal of the Chem. Soc. LVII. 1890. p. 458.

[4]) Jahrb. f. wiss. Bot. 31. 1898. p. 599.

[5]) Traité de Microbiologie II, 1899, pg. 84—88.

to observe, that a starch-saccharifying enzyme is secreted when starch and dextrin are given as carbon food, but furthermore also with maltose, glucose, glycerin, lactic acid, malic acid, and acetic acid, only the amount of enzyme is by no means always equally great. The sugar thereby resulting, was identified by the osazone in the case where the enzyme was produced in a glycerin-liquid; here, too, it was d-glucose. Presently it will be shown why this is of importance.

On the other hand, the inversion of cane-sugar or the hydrolysis of maltose can be very exactly determined. I therefore fixed my attention on these two conversions and in particular on the latter, because it was soon evident that invertase is secreted in all the examined cases, albeit not always in equally large quantities (i. e. when as carbon food were used cane-sugar, maltose, glucose, glycerin, lactic acid, malic acid, and acetic acid). Quite different is the case with the maltose enzyme, which I will give the name of *maltoglucase.*

As is known, an enzyme forming glucose, has been named glukase by BEIJERINCK and the German investigators. If the view of CROFT HILL [1]) that this conversion is a reverse action proves to be right, this name already gives rise to confusion, still more, however, if one and the same plant, as Monilia sitophila, secretes two enzymes, both forming glucose, one from dextrin (starch), the other from maltose. The nomenclature of DUCLAUX and his school would be "maltase", but here we find the same difficulty, for starch is not always converted in the same way by different enzymes; would it then be correct to speak of amylase in every case? The confusion becomes still greater by the fact that maltase is quite another thing for DUCLAUX than for BEIJERINCK and WIJSMAN. In my opinion the problem is best solved by using a double name and thus to speak of maltoglucase. The same nomenclature can be used in all cases where the product of the conversion is well known and simple.

Maltoglucase now (with a single exception of which presently more), is exclusively secreted at the nutrition of Monilia sitophila with certain carbohydrates, and that in a very unequal degree. The following non-carbohydrates, when serving as carbon food, give no rise to the secretion of the here meant enzyme: glycerin, erythrite, mannite, dulcite, isodulcite, sorbite, ethyl-acetate, acetic acid, lactic acid, malic acid, succinic acid, citric acid, glycocol,

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[1]) Journal of the Chem. Soc. 1898, p. 634.

asparagin and tyrosin. In this list we find, among others, glycerin; in this liquid, in which no maltoglucase is produced, the amylolytic enzyme is found, and it is worth mentioning that it causes the production of glucose from the starch. This proves that here DUCLAUX's [1]) view is untenable, according to which in all cases, where, by the action of enzymes glucose takes rise from starch, there would first be formed maltose, which then, by another enzyme would be converted into glucose. Nor is the opinion of BEIJERINCK [2]) tenable in this case; his glucase would convert as well maltose, as erythro- and malto-dextrin into glucose. Hence we must admit that here the conversion into glucose is effected, either by a single enzyme, or by two enzymes, one of which converts starch into dextrin and perhaps corresponds with one of the constituents of diastase (i. e. the dextrinase of WIJSMAN [3]), the other hydrolysing dextrin into glucose.

Neither does Monilia sitophila secrete malto-glucase at nutrition with the following carbohydrates: arabin, l-arabinose, lactose and inulin (when Ammonium salts or nitrates serve as source of nitrogen). Here it should be borne in mind that my meaning is of course: no measurable quantities of maltoglucase. As the most accurate measurements may be done by means of the polarimeter I have used this instrument and have then considered changes of rotation below 0.10° as to lie within the limit of errors. Only arabinose lay about near this limit, but if this might point to the secretion of traces of enzyme, it could still be attributed to impurities. That these can indeed be of influence, was for instance shown with lactose. Pure commercial milk-sugar gave rise to the secretion of small quantities of enzyme (when a 5 pCt. solution was used the decrease in rotation was 0.36° in 3 days), but after I had purified it and then repeated the experiment no enzyme was secreted anymore.

Large quantities of maltoglucase are secreted, when the mould can use, as source of carbon, first of all raffinose or maltose, further, commercial dextrin or starch. In less degree cellulose gives rise to the secretion of the enzyme; still less galactose, xylose, glycogen, whilst last of all, come cane-sugar and d-fructose. With the last mentioned carbohydrates, peptone stands about on a level, whilst also in milk a slight quantity of enzyme is secreted; in this latter

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[1]) Traité de Microbiologie. II. 1899, p. 471 vlg.

[2]) Centralbl. f. Bakter. u. Parasitenk. 2e Abth. I. 1895, p. 221.

[3]) De diastase beschouwd als mengsel van maltase en dextrinase. Amsterdam 1889.

case the cause cannot be sought in the lactose or the fat, so that here, too, the proteids of the milk must cause the secretion of the maltoglucase. Would not the carbohydrate-rest, which probably occurs in the proteid molecule, explain this fact?

It is in general the best-feeding carbohydrates, which cause the secretion of the greatest quantity of enzyme, but this does not include that there should be a direct relation, as proved by the following data:

Carbon food.	Relative quantity of secreted enzyme.	Quantity of mould obtained (dry matter).
10 pCt. raffinose	10 17	257 mGrs.
5 » dextrin	7.17	61 »
2,5 » maltose	5.14	41 »
5 » galactose	0.68	12 »
5 » glycogen	0.55	36 »
5 » cane-sugar	0.26	21 »
5 » lactose	0	30 »
5 » peptone	0.50	124 »

Another question to be answered was, whether, at the nutrition with the same substance but in varying quantities, there exists a direct relation between the quantity of the food and that of the secreted maltoglucase.

For the measurement of the relative quantity of enzyme, there are two ways: one is to observe how much time is required to convert equal quantities of the substance; the quantities of enzyme are then inversely proportionate to those times. Or, the quantities of substance, converted in equal times, are determined; in the beginning of the reaction these quantities are proportionate to the quantities of the enzyme. I have used the latter method after first having convinced myself of its usefulness by some preliminary experiments.

The result of a series of experiments, taken in particular with raffinose, but also with maltose, was that the quantity of secreted enzyme rises with the amount of sugar given as food; so long as the latter is still present in a slight quantity, both increase almost proportionately. But as the concentration of the solution becomes greater, the increase of the secreted enzyme is seen to diminish, until it reaches a maximum, then to decrease at still higher concentration of food. This maximum lies for raffinose and maltose at a concentration of about 10 pCt.

Very possibly the idea might arise that in these strong raffinose and maltose solutions, the quantity of secreted enzyme becomes smaller by the great osmotic pression of the solution; this is not however the cause. In order thereabout to get certainty, I have mixed the raffinose and maltose solutions with dilute glycerin of such a strength that all solutions of varying sugar amount were isotonic. Glycerin was taken, because, as said above, it has no influence on the secretion of maltoglucase, neither does it act accelerating or retarding on the reaction of the enzyme, at least not in the used concentrations (as shown by other particular experiments). It was thus proved that under these conditions the quantity of secreted enzyme mounted likewise with increasing concentration, about to the same maximum; only the proportionality at feeble concentrations was sometimes less striking than in absence of glycerin. This is probably a consequence of the more vigorous development of the mycelium of Monilia when, together with the slight amount of raffinose or maltose, glycerin was also present, which fact was already briefly discussed above.

The question arises whether the different amounts of secreted enzyme, cannot be a consequence of the degree of development of the mould. For it might be thought that each cell of the mycelium, so long as it lives, secretes a certain constant quantity of enzyme, hence, that the more vigorously the mycelium has developed, the more enzyme will be secreted. I have tried to answer this question by also weighing in every case the crop of mould obtained (after drying). I will give one of the series of figures thus obtained.

In column I is found the constitution of the nutrient liquid, in column II the crop of mycelium in mgrs., in column III the quantity of secreted enzyme, whilst column IV indicates the quantity of enzyme secreted on 100 mgrs. of dry matter of the mycelium.

	I.						II.	III.	IV.
1.	0	pCt.	raffinose	3.27	pCt.	glycerin	25	0	0
2.	0	»	»	3.27	»	»	21	0	0
3.	0.16	»	»	3.24	»	»	141	0.32	0.23
4.	0.31	»	»	3.22	»	»	116	0.24	0.21
5.	0.62	»	»	3.16	»	»	208	0.57	0.27
6.	1.25	»	»	3.06	»	»	211	1.03	0.49
7.	2.5	«	«	2.86	»	»	230	1.77	0.77
8.	5	»	»	2.46	»	»	257	3.16	1.23
9.	10	»	»	1.63	»	»	342	3.87	1.13
10.	20	»	»	0	»	»	528	3.74	0.71

When considering only the figures of rows 3, 4 and 5 in column IV, they are rather alike, but further there hardly appears any relation between the development of the mycelium and the quantity of secreted enzyme. Though it will not be possible to make a pure comparison, as then for the total weight of mould obtained allowance should be made for the portion present in the air, the dead cells, etc., still rows 9 and 10 show that the mass of mycelium can increase considerably (and here in both cases all was nearly completely immersed) whilst the quantities of secreted enzyme have remained rather unchanged.

Whilst we saw already that the nature of the food is of great influence on the secreting or not secreting of maltoglucase, it is now evident that the quantity of the food offered, likewise exerts influence on the quantity of secreted enzyme, in such a sense, that both increase conjointly, but that very great quantities of food act prejudicially on the secretion of the enzyme.

There is a certain disposition to admit that the secretion of enzymes in general would be the consequence of the want of certain nutrients, and would indicate, as it were, a hungry condition of the cell. The investigations here communicated do not agree with this view; they contain a warning against too rashly drawing conclusions on this head.