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greatest for the perihelion, smallest for the aphelion. As a mean value of all these times we may consider

$$T_1 = T\left(1-\frac{3a}{2p}\right).$$

For this time KEPLER's third law becomes

$$\left(a-\frac{ae^2}{1-e^2}\right)^3 : T_1^2 = \frac{a}{8\pi^2}.$$

This deviates from KEPLER's law less than (43).

Chemistry. — "*The Metabolism of Aspergillus niger.*" By Dr. H. J. WATERMAN. (Communicated by Prof. J. BOESEKEN.)

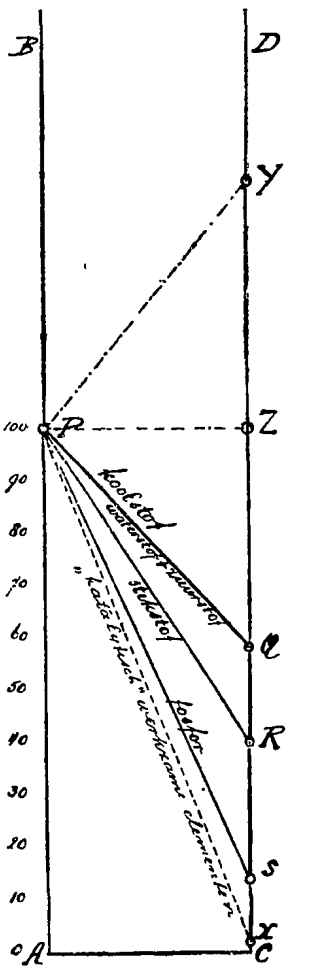
(Communicated in the meeting of May 27, 1916.)

In researches described in previous communications [1]) I have demonstrated that the quantity of different elements accumulated in the cells of *Aspergillus niger* is subject to very great variations. The investigation was carried out quantitatively for carbon, nitrogen and phosphorus and qualitatively for the element sulphur Whereas from 100 parts by weight of carbon, assimilated as glucose, 55 parts, for instance, are absorbed after three days in the fungous material, this after 21 days amounts to only 31, so not quite 60% of the quantity originally taken up. The same applies to the nitrogen and particularly to the phosphorus in a still higher degree.

The quantity of nitrogen present in the cells falls in course of time to $^3/_5$—$^1/_3$ of the quantity present in the young cells and with phosphorus even to $^1/_7$—$^1/_{10}$. Also the sulphur is accumulated in young cells. On increasing age the superfluous quantities of the said elements are excreted.

On account of experimental difficulties I have given up the idea of determining the progressive course of the hydrogen and the oxygen separately, but have now decided to calculate the sum of these two elements accumulated in different periods in the fungous material. For, if we know the quantity of dry substance, likewise the percentage of carbon, nitrogen, phosphorus and ash, it is possible to determine with sufficient accuracy for my purpose the joint amount of hydrogen and oxygen.

[1]) Folia microbiologica, Holländische Beiträge zur gesamten Microbiologie I, 422 (1912); These Proceedings November 30 (1912) p. 753, February 22 (1913) p. 1047 and 1058, March 22 and April 25 (1913) p. 1349; Handelingen XIVe Ned. Natuur- en Geneesk. Congres p. 125.



After 4 days. After 21 days.

koolstof = carbon
waterstof + zuurstof = hydrogen + oxygen
stikstof = nitrogen
fosfor = phosphorus
katalytisch werkzame elementen = catalytically active elements

I have noticed that just as in the case of carbon, the quantity of hydrogen and oxygen present in a young fungous film diminishes in course of time and after 21 days has already fallen to about one half of the original quantity. In the figure this is represented graphically. On the vertical axis AB is indicated the quantity of the elements present in a young fungous film obtained under definite circumstances. This quantity is, of course, very unequal with different elements, large with carbon, small with phosphorus. If we assume that AP represents the quantity of each of the elements concerned in a young fungous film (4 days old), CQ, CR, CS etc. represent, respectively the quantity of carbon and hydrogen + oxygen, nitrogen, phosphorus etc. present in the 21 days old fungous film. $CQ = \pm {}^1/_2 AP, CR = \pm {}^2/_5 AP, CS = \pm {}^1/_8 AP$.

The lines PQ, PR and PS then represent the diminution of the quantity of the correlated elements when the fungous material gets older. As demonstrated previously [1]) they are in reality not straight but curved.

The utilization of the intermediate products present in the young cells in which process carbon dioxide, ammonia, phosphate, sulphate etc. are formed, explains the entire phenomenon. It is remarkable that many of the excreted products can again serve as nutriment so that for instance a small quantity of phosphorus can participate a few times in the metabolism of many cells.

I already expressed my opinion some time ago that there are also elements that will exhibit this phenomenon in a still higher degree than the phosphorus. These then go and resemble catalytically active elements (line PX). That the accumulation phenomenon does not remain confined to the elements carbon, hydrogen, oxygen,

[1]) Purposely I have united here the said lines in a graphic representation because they are related and descend *simultaneously*.

nitrogen, phosphorus and sulphur is shown from the determination of the sulphated ash from the fungous material at different periods of the development.

50 cc. of a nutrient liquor of the composition: tapwater, 2% anhydrous glucose, 0.3% NH_4NO_3, 0.3% KH_2PO_4 and 0.2% of crystallised magnesium sulphate was inoculated with traces of *Aspergillus niger*. With the fungous material obtained after 47 days at 32°—33°, which was then washed with distilled water, a sulphated ash determination was made. All the glucose was used up. I obtained in four cases 5, 5, 5.5 and 6 mgs of ash, respectively (table 1). Such a trifling quantity of ash made us already expect that the elements occurring in the sulphated ash would be present in young cells in larger quantities than in old ones. The proof therefore is given by the further experiments mentioned in table I.

TABLE I.

Nutrient liquor: 50 cc. of tapwater wherein dissolved 2 % anhydrous glucose, 0.15 % NH_4NO_3, 0.15 % KH_2PO_4, 0.1 % crystallised magnesium sulphate. Inoculated with *Aspergillus niger*. Temperature during cultivation $^{32}/_{33}$° C. Quantity of ash [1]) in mg., after moistening the fungous material with strong sulphuric acid [2]).

Number of days after inoculation.				
Four	*Nine*		*Sixteen*	*Forty seven* [3])
16	9	9	7	5
15	8	9		5
18	9	10		5,5
	11	9		6

On the ground of the above it was to be expected that if we remove a young, only just formed, fungous film from the nutrient liquor and place it on distilled water after having first washed away the adhering fluid, the utilisation of the intermediate products will go on to a considerable degree if, at least, the temperature does not alter (32°—33°). Yet the matter was not quite so simple as I suspected. The possibility existed that one or more of the elements are accumulated not in the young but in the old cells

[1]) Ash entirely free from carbon.

[2]) The figures with the same number of underlines belong to the same experimental series.

[3]) In these experiments the inorganic nutriment was: 0,3% NH_4NO_3, 0,3% KH_2PO_4 and 0,2% crystallised magnesium sulphate.

(line $P\ Y$). In this case the element, whether added purposely or not ought to be present in the liquid. At a possible non-presence the ordinary utilisation of the intermediate products with the other elements would be retarded or even not take place at all, at least if the said element should have an *essential* and not a subordinate significance for the metabolism. Experiments in connexion herewith have, however, shown that such an element does not exist (See Table II).

TABLE II.

50 cc. of nutrient liquor composed of tapwater in which is dissolved 2 % anhydrous glucose, 0.15% NH_4NO_3, 0,15% KH_2PO_4, and 0.1% crystallised magnesium sulphate. Temp. 33°. Inoculated with *Aspergillus niger*.
Dry substance in mg (dried at 105° to constant weight).

After 4 days [1])	After 5 days [1])	After 5 days [1]) of which the last day on dist. water	After 7 days [1]), of which the last three days on dist. water
436	367	368	341
439		353	314
			315

For the utilisation of the intermediate products and the consequent decrease of the quantity of dry substance an absorption of any element from the nutrient liquor is thus no longer essential [2]) in the normal metabolism of *Aspergillus niger*. All elements needed for the metabolism of *Aspergillus niger* are accumulated in the young fungous material and when this gets older they are partly excreted [3]).

Elements that are permanently accumulated, whilst the cells are growing older, do not exist.

Dordrecht, May 1916.

[1]) All the glucose is consumed.

[2]) The element oxygen is excluded here.

[3]) Represented in the Fig. by the lines PQ, PR, PS, PX etc PZ is a particular but improbable case.