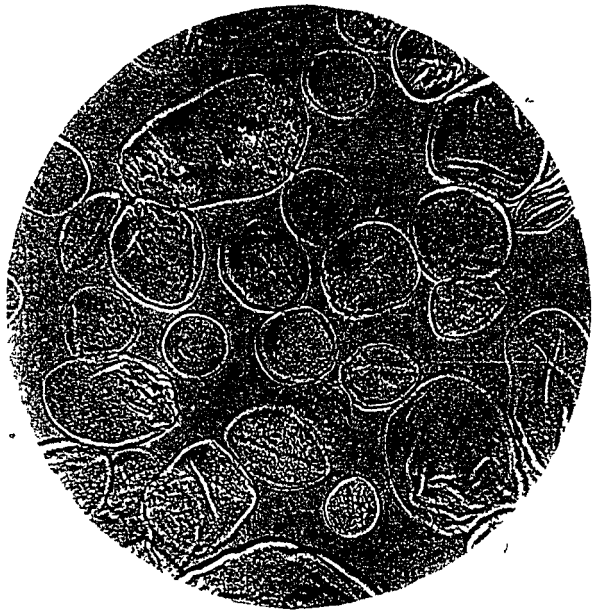
Botany. — *"Structure of the starch-grain"*. By Prof. Dr. M. W. BEIJERINCK.

If one gram of potato-starch is boiled with 100 cM^3 of distilled water this is just sufficient to bring the grains to their maximum of swelling and make the starch take up about 70 % of the water so that it remains suspended and cannot precipitate, as the swollen grains touch one another. Each grain swells thereby to a somewhat irregular globule whose diameter is about 3.5 times that before the ebullition. Whether the boiling lasts shorter or longer is of no consequence. If more water is used for the boiling no further swelling takes place; when left to sedimentation the liquid above the starch colours but feebly blue with iodine.

When a microscopic preparation is made, containing but few starch-grains, and a strong tannin solution flows sideways under the cover-glass, the following is seen (compare the figure).



EXPLANATION OF THE FIGURE.

Magnified 200 times.

Potato-starch after prolonged boiling and treatment with a tannin solution The grains are by the boiling changed into little vesicles with dissolved contents. The wall of the vesicles consists of amylocellulose (amylopectose), the contents of granulose (amylose), the latter being precipitated by the tannin.

At the moment the tannin comes into contact with them, the grains, which at first sight seem homogeneous, show a very distinct membrane through which the tannin easily diffuses to the inside where it directly forms a characteristic precipitate. When using a more

dilute solution [1]) this precipitate consists of little droplets in very lively Brownian movement and with a more concentrated tannin solution, of solid particles, adhering together and filling up the whole inner space of the vesicle. This experiment is so simple and convincing that it cannot be doubted for a moment but the boiled starch-grain consists of a solid, sac-shaped, quite closed wall, containing a liquid.

How it is possible that this fact seems unknown I cannot understand, but I have nowhere found it mentioned in the extensive literature about this subject.

The liquid in the vesicle is a granulose solution, or as is said at the present day, an amylose solution, containing 0.6 gram of the 1 gr. originally used, which diffuses but with difficultly through the walls into the surrounding water. If, however, the boiled starch is rubbed fine with sand the delicate sacs burst and the granulose solution diffuses in the water, which then becomes intensely blue with iodine.

That the wall consists of a very soft substance may be observed as well by its great variability of shape at pressure, as by the ease with which it is distended to short threads by moving the cover-glass, to which it adheres, when touched by it. When the boiled starch-grains are washed out during some days with water constantly renewed, it is possible finally to obtain the vesicles without their contents and filled with water only; after drying they weigh 0.4 gr. if one gr. of starch has been used. With iodine they colour lighter than the granulose and somewhat violet. When preserved they become partly soluble in water containing chloroform. By leukodiastase they are easily converted into maltose and dextrine, quite like granulose; by erythrodiastase a little less easily, but a marked difference does not exist. [2])

If the boiling is effected not in distilled but in canal water, the starch shows a strong disposition to precipitate whereby after 24 hours a layer results of $^1/_3$ to $^1/_4$ of the whole volume if again 1 % starch is used. If 4 % starch or more is boiled in canal water

[1]) Very much diluted tannin solutions give no precipitate at all with starch- or granulose solutions.

[2]) By leukodiastase I understand the slowly diffusing diastase secreted by the germs of germinated corn-grains, which on starch-gelatin plates, when treated with iodine, produces diffusion fields which remain uncoloured. By erythrodiastase the more quickly diffusing diastase of the endosperm of the grain, which on the said plates after treating with iodine, is recognisible by the erythrodextrine reaction. WIJSMAN (De diastase beschouwd als mengsel van maltase en dextrinase, Amsterdam, 1889) called leukodiastase "dextrinase" and erythrodiastase "maltase", but these terms are not well chosen.

no sedimentation at all occurs, the swollen grains again touching one another. The precipitation may be caused in the starch boiled with distilled water by addition of dilute solutions of salts, acids, or alkalies. At 0,001 % a slight contraction of the vesicles is already visible and it reaches its maximum at about 0,1 %. [1]) With still stronger concentrations an accrease in thickness of the precipitated layer is observed, probably because the vesicles then lose somewhat of their weight in the heavier liquid. [2]) As non-electrolytes such as cane sugar, urea, aethylalcohol, and methylalcohol, even in 1 % solutions, cause no sedimentation at all, it is evident that we have to deal here with an ionreaction, which perhaps will prove to be very well apt for exact measure. Aethylalcohol of 5 % and methylalcohol of 6 %, however, distinctly bring the vesicles to precipitation; but then the superstanding liquid becomes rather turbid, the dissolved granulose precipitating also. Above 10 % methylalcohol the precipitation is complete.

If the starch is boiled in dilute salt solutions, the volume of the sediment after standing is as large as if the salt had been added later to the starch boiled in distilled water.

When the sedimentation is caused by ammonium sulphate it is easy to show that as well the ammonium as the sulphuric ion are present in stronger concentration in the precipitate than in the liquid above it.

The foregoing is quite in accordance with the results of an investigation of Mme Z. GATIN-GRUZEWSKA. [3]) By extraction of starch with dilute caustic soda she obtained a soluble substance, amylose, and an insoluble rest of amylopectose in about the same proportion as the above (0.6 and 0.4). Her view, however, of the localisation of the two constituents is another than that which follows from my observations.

She says that amylopectose forms little scales or sacs, evidently corresponding with the layers of the starch-grain, so that this constituent would occur as well within as outside the grain, whilst, according to my experience, the whole inner portion dissolves in boiling water and is homogeneous, the outerwall only being insoluble and thus materially different.

[1]) No great difference in the thickness of the precipitated layer (ca. 4 cm. from a liquid layer of 17 cM.) was perceptible after 24 hours at room temperature when using 0,1 % K_2HPO_4, KCl, NaCl, $(NH_4)_2SO_4$, $CaCl_2$, Al_2Cl_3, KNO_3, HCl, or Na_2CO_3.

[2]) More dilute solutions of sugar and urea do cause some sedimentation for a not yet explained reason. Stronger solutions do the same perhaps because of contamination by electrolytes.

[3]) Comptes Rendus T. 146 p. 540, 1908.

The words "amylose" and "amylopectose" have first been used by L. MAQUENNE and E. ROUX, [1]) but they consider both these substances as perfectly mixed and say: "L'empoix d'amidon est constitué par une solution parfaite d'amylose, épaissie par l'amylopectose" (l. c. pag. 219).

That MAQUENNE, even after the communication of Mme GATIN-GRUZEWSKA, had by no means the view here given follows from the observations which he adds to the said communication. [2])

The change of the terms "amylocellulose" and "granulose", so long existing in the literature, into "amylopectose" and "amylose" by MAQUENNE, seems not necessary.

The difference between the walls and the contents of the starch-grain probably reposes on incrustation. We have namely to think the surface of the grain as consisting of the albuminous matter of the amyloplast mixed with the secreted granulose by which the thus formed mixture has become insoluble in boiling water. This would be in accordance with the general observation, that incrusting substances highly alter the solubility of bodies susceptible of imbibition, of which the lignified and suberified cell-walls of plant cells and tanned leather are good examples. This conception would lead to the conclusion that the amyloplast does originally incrust the membrane of the starch-grain, but later draws back from it, wherewith the change of amylocellulose (amylopectose) into granulose (amylose) would correspond.

If this view is right the quantity of albuminous matter, which occurs in the membrane, must be very small, for in the rate of nitrogen no distinct difference between amylocellulose (amylopectose) and starch could be found, in both cases it being about 5 milligrams per 100 grams of dry matter.

MAQUENNE says that his amylopectose is not coloured by iodine; the amylocellulose (amylopectose) obtained from starch after extraction of the granulose (amylose) in the manner here described, proves to colour violet blue with it. It is not impossible that in this case, too, a kind of incrustation should occur, namely of an adsorption of granulose in the amylocellulose wall, which then itself would in pure condition remain uncoloured by iodine.

All other species of starch examined by me behave in the same way as potato-starch.

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[1]) Recherches sur l'amidon et la saccharification diastasique. Ann. d. Chimie et de Physique, 8e Série, T. 9, pag. 179, 1906.

[2]) Observation sur la Note de Mme GATIN-GRUZEWSKA. Comptes Rendus T. 106 p. 542, 1908.