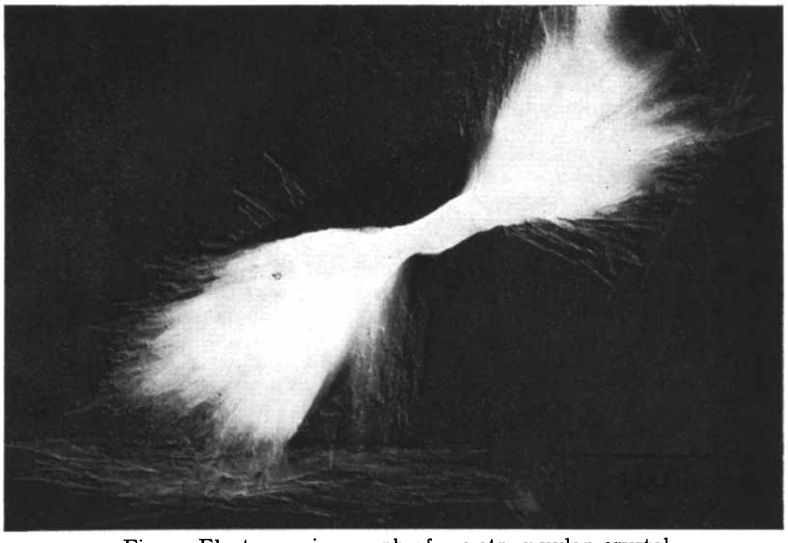

Fig. 1. Electron micrograph of rye straw xylan crystal.

of ZECHMEISTER AND TOTH[3]. Apparently, the crystal structure of xylan is different from those of cellohexaose and cellulose II, which are similar; (the sphero-crystals of cellohexaose are negatively birefringent, which suggests tangential molecular orientation, since a flat helical molecule form is excluded here).

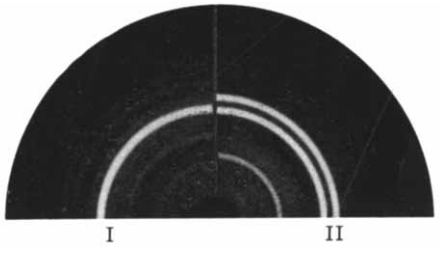

Fig. 2. Quadrants of X-ray powder diagrams of xylan (I) and cellohexaose (II). Specimen-film distance 40 mm.

ACKNOWLEDGEMENTS

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ON THE NATURE OF FLORIDEAN STARCH AND *ULVA* STARCH

by

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Floridean starch, the reserve substance of some of the red algae, has been studied by several investigators. It is still an open question, however, to what extent this material may be regarded as a true starch, comparable to that of the higher plants. KYLIN's observation[1] that floridean starch

from *Furcellaria fastigiata* is hydrolized by β-amylase (malt diastase) provides good evidence for the idea that "normal" 1–4 starch linkages are present between the glucose residues in this material. On the other hand, BARRY *et al.*[2] have observed a pronounced resistance against attack by crystalline β-amylase in starch from *Dilsea edulis*. This fact and the results of certain periodate oxidation experiments led the latter authors to believe that a considerable proportion of the linkages in this "starch" must be of the 1–3 type. Prompted by his interest in paramylon, an algal reserve substance in which the presence of 1–3 linkages is very likely[3], the senior author has isolated native (though iodine stained) starch from 4 *Odonthalia* species, namely *O. kamtchatkensis*, *O. lyalli*, *O. washingtonensis* and *O. floccosa*, by a process of fractionated sedimentation. *Furcellaria* and *Dilsea* were not available in the Puget Sound region where these collections were made. Starch from the green alga, *Ulva expansa*, was collected for comparison.

With a view to the surprising information obtained from an X-ray examination of *Euglena* paramylon (KREGER AND MEEUSE[3]), it seemed wise to subject the *Odonthalia* and *Ulva* starches to such an examination, prior to a complete chemical and enzymic analysis. The results of our observations are as follows:

The X-ray diagrams of the *Odonthalia* starches (Fig. 1, I) reveal that these materials must indeed be true starches. The diagrams correspond very closely with those shown by most tuber starches such as the diagram of potato starch in Fig. 1, II. The differences between the starches of the *Odonthalia* species were small. The weakest interferences were given by *Odonthalia floccosa* starch in which the double refraction of the granules also appeared to be weakest.

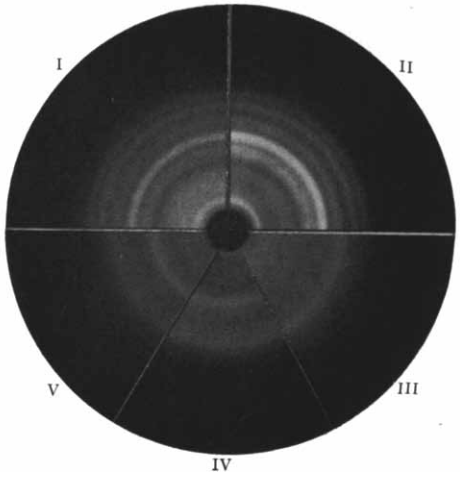

Fig. 1. Quadrants and sectors of X-ray powder diagrams of starches from *Odonthalia kamtchatkensis* (I), potato (II), *Ulva expansa* (III) and from alcohol precipitated starch from *U. expansa* (IV) and potato (V).

The main difference from starches of higher plants is that *Odonthalia* starch grains do not easily form a colloidal solution. The present authors do not believe that this behaviour of the *Odonthalia* starch grains can be ascribed to the fact that they had undergone an iodine treatment.

With some difficulty, colloidal solutions of *Odonthalia* starch could be obtained by a treatment involving the use of hot, neutral, concentrated solutions of calcium chloride. In these solutions, malt β-amylase of proven purity effected about the same degree of breakdown (38%) as it did in glycogen solutions of the same concentration. This suggests a high degree of ramification in the floridean starch. The same conclusion can be drawn from measurements of the "blue value" after staining of the substance with iodine.

Under identical conditions, the rate of breakdown of floridean starch from *Odonthalia* by α (salivary) amylase is only slightly different from that of starch or glycogen by the same enzyme. Likewise, the results of a periodate oxidation carried out according to the directions of BARRY *et al.*[2] are almost the same for glycogen and for the floridean starch; resistance against the oxidant is slightly higher than it is in potato starch. A more detailed account of these chemical and enzymical experiments will be given elsewhere. The results seem to be in fair agreement with those of O'COLLA[4], who independently has made a renewed chemical study of *Dilsea* starch. This author has recorded a 48% hydrolysis by (crude) β-amylase and mentions some resistance against periodate attack. His idea that floridean starch might be related to laminarin, the reserve polysaccharide of some brown algae, is disproved by the X-ray diagram of *Odonthalia* starch, as is the suggested analogy with lichenin (BARRY *et al.*[2]).

The starch of *Ulva expansa* consists of very small grains (2–5 μ). It is remarkable in that it shows no appreciable birefringence, whereas the *Odonthalia* grains (3–9 μ) exhibit a strong spherite cross between crossed nicol prisms. The starch dissolves readily in boiling water and forms no paste. The solution is stained blue by iodine.

The X-ray diagram does not resemble any of the known types of starch diagrams. It shows one ring of moderate intensity, standing out against a diffuse background (Fig. 1, III).

The absence of birefringence and the deviating X-ray diagram suggest that the starch is more different from that of higher plants than floridean starch is. Evidence that we are nevertheless dealing with a starchlike product was obtained in the following way. If the warm solution of the substance in water is treated with ethanol, a white precipitate is formed, as is the case with normal starches. The X-ray diagram of the dried precipitate (Fig. 1, IV) shows 3 rather diffuse rings, which in diameter and intensity correspond with those of a control prepared from potato starch in the

same manner (Fig. 1, V). It may be noted that the ethanol precipitation was carried out according to BEAR's method[5] for the preparation of the "V" modification of starch with sharp X-ray interferences. We did not succeed, however, in obtaining such sharp interferences as shown by BEAR's diagrams.

A more detailed chemical study of both these starches is under way.

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PARTIAL PURIFICATION OF DNASE II FROM THYMUS*

by

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The abbreviations used in this paper follow the pattern suggested previously[1]. The first evidence that thymus contains a nucleodepolymerase (DNase II), which is different from the desoxyribonuclease of pancreas (DNase I), was obtained by MAVER AND GRECO[2]. Their findings were almost simultaneously confirmed by WEBB[3], and in this laboratory[4]. Furthermore, it was found[4] that the intracellular distribution of this enzyme does not coincide with the distribution of its substrate (DNA). The latter conclusion was confirmed by SCHNEIDER AND HOGEBOOM[5] on liver cells, by ALLFREY AND MIRSKY[6] on a number of tissues, and by WEBB[7] on thymus. WEBB[8] described a method for partial purification of the DNase II from thymus.

A method, devised in this laboratory, and leading to a considerable purification of the DNase II from thymus is described. The extraction procedures in both WEBB's and our methods are almost identical. Several other methods of extractions have been tried but none was found better[9]. Contrary to WEBB[8] we were unable to detect any increase of activity after 24 or 48 hours of autolysis either at pH 5, or in an unbuffered extract.

Step 1. Fresh calf thymus (5 lbs) is blended in portions in the Waring blendor and is extracted overnight with 11 liters of ice-cold 0.85% NaCl containing 0.02 *M* $CaCl_2$, after the pH of the mixture has been adjusted to 5.0. The mixture is strained through four layers of gauze, the cloudy liquid is mixed with 250 g of Celite No. 545 and is refiltered through a layer of Celite No. 512 (300 g) on 32 cm Whatman No. 1 paper**. The enzyme is precipitated from the clear filtrate between 30 and 90% saturation of $(NH_4)_2SO_4$.

Step 2. The precipitate from the Step 1 is dissolved in water, the pH is adjusted to 2.5 and the concentration of protein is adjusted to give an optical density reading, $E_{280}^{1\,cm}$, of 5.0 to 7.0. The proteins are fractioned at this pH at 40, 60, and 80% saturation. The third precipitate (between 60 and 80% saturation) contains most of the activity.

Step 3. This precipitate is dissolved in 0.1 *N* acetate buffer pH 5.5, protein concentration is adjusted to $E_{280}^{1\,cm} = 10$, and pH to 5.5. The precipitate obtained between 50 and 80% saturation of $(NH_4)_2SO_4$ contains most of the enzyme.

Step 4. The precipitate from the step 3, combined from three or four preparations, is dissolved in water and is dialyzed for 48 hours against 0.01 *M* sodium acetate pH 4.0. The protein concen-

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** On a large stainless steel Büchner funnel, Model 503, a gift from the American Biosynthetics Corporation, Milwaukee, Wisconsin.