MACROMOLECULES IN CELL STRUCTURE

A. FREY-WYSSLING

MACROMOLECULES IN CELL STRUCTURE

A. Frey-Wyssling

Harvard University Press, Cambridge, Massachusetts

Distributed in Great Britain by
Oxford University Press, London

PRINTED IN NORTHERN IRELAND

Preface

The submicroscopic structure of protoplasm, as outlined in my monograph with that title (Elsevier, Amsterdam, 1953), has become a vast field of investigation in biophysics. It is no longer possible to give a complete survey of its progress in a short course. Therefore, only a few highlights and recent trends of this modern science were chosen for presentation in the Prather Lectures which I delivered at Harvard University and on which this book is based. Since it is the task and the privilege of a lecturer to present his own views and experiences, I have emphasized the problems that we have tried to solve in our Department of General Botany at the Swiss Federal Institute of Technology in Zurich.

I should like to thank my associates P. D. Dr. K. Mühlethaler, P. D. Dr. F. Ruch, and Dr. H. H. Bosshard, as well as my former assistants, Dr. E. Steinmann, Dr. A. Vogel, and H. Stecher, for their valuable coöperation. Likewise, I am deeply grateful to those colleagues who have put additional electron micrographs at my disposal.

At the same time I wish to express my thanks to the officers of the Harvard Department of Biology and to the Prather Lecture Committee for their invitation to present my subject before a distinguished audience.

A. FREY-WYSSLING

Zurich, February 15, 1956

Contents

Introduction

"The Domain of Neglected Dimensions"

In 1915 Wolfgang Ostwald published an attractive booklet with the sensational title *Die Welt der vernachlässigten Dimensionen*, i.e. "The Domain of Neglected Dimensions" (Ostwald 1915). It contains five lectures on colloid chemistry delivered at several universities in the United States shortly before the first World War. The unusual title of this publication shows that only 40 years ago there was still an animated discussion on the size and the nature of colloidal particles of which the existence was proved by such ingenious indirect methods as ultramicroscopy, ultrafiltration, and ultracentrifugation.

Since the microscopic constituents of the cells had been recognized as colloidal systems, the cytologists had tried to apply these new methods to living systems, though with rather poor results. They found that cytoplasm, nuclei, and chloroplasts were "optically empty" (Guilliermond, Mangenot, and Plantefol 1933) and that the cell had to be considered as "a tiny sac of fluid in motion" (Heilbrunn 1930). It was not until 1938 that it was realized that the disperse character of their constituents alone could not explain morphogenesis, shape, and orientated growth of cytological objects, but that the interrelation and the gel formation of these colloidal particles is of paramount importance (Frey-Wyssling 1938). So research on the gel structure and the reversible sol-gel transformation of protoplasm and its derivatives has become a fascinating goal of cytology. I have called this new field of investigation *Submicroscopic morphology*. In the early days we used polarizing microscopy for this research (Ambronn and Frey 1926), but soon x-ray diffractometry brought welcome support (Frey-Wyssling 1937*a*), and since 1944 electron microscopy (Frey-Wyssling and Mühlethaler 1944, 1946) has raised submicroscopic morphology to a generally recognized standard science.

The ultimate aim of this new science is to establish the shape and the arrangement of the macromolecules in cytological objects. In so doing, the existing gap between chemistry and morphology can be bridged. This is urgently necessary, since the basic biological sciences have separated

into a morphological and a chemical branch, which in medicine are called histology and physiological chemistry. When I was a student the representatives of those sciences no longer understood one another. The biochemist ironically called the histologist a stamp collector because his purpose was to produce and assemble flawless preparations. The histologist, on the other hand, thought of the biochemist as a poor biologist, because he mixed up all the cell constituents of a tissue, made a chemical analysis of this awful mixture, and drew whimsical conclusions from such a mean result.

The situation of that time is best illustrated by the following anecdote, related by Professor A. V. Hill (London): A neurochemist called a homogenate of brain which he studied "the natural brain," and only after having added cyanide did he consider it to be a "denatured brain." Of course, for a real biologist a natural brain with its complicated microscopic and submicroscopic structures is quite another thing than such a tissue *Brei*.

Fortunately this lack of reciprocal understanding and appreciation between morphologists and biochemists is rapidly diminishing today. The biochemist is becoming increasingly interested in the morphology of macromolecules revealed by the electron microscope, and the morphologist is incited by the tentative results to find out the internal chemical structure of those macromolecules. So the antagonism between morphology and chemistry, which has for decades divided biology into two hostile camps, no longer exists. Owing to the development of submicroscopic morphology, which clarifies the domain of hitherto "neglected dimensions," we can foresee a welcome unification of the two separate basic sciences in biology.

Our scope is thus to trace the structure of our cytological objects starting from the well-known amicroscopic organic micromolecules, which constitute the visible submicroscopic macromolecules, through the whole colloidal range to the microscopic constituents of the cell. This seems an almost impossible task, since the number of these particles in a cell is incredibly high and there are so many possibilities of arranging micro- and macromolecules.

If we admit a not yet vacuolated cubical meristematic plant cell of 35-μ diameter and 10 percent dry matter, it contains about 100 billion (10^{11}) macromolecules of the Svedberg protein unit size (molecular weight, 17,600; diameter, 35 A) or 15 trillion micromolecules of the average amino acid size (molecular weight, 122). By way of comparison, in a

cubic room 35 m on a side, the "macromolecular particles" would measure only 3.5 mm in diameter! Our task would then be to establish the internal structure of these particles and their exact arrangement in the available space of the enormous room. It is evident that there are so many possibilities of filling the room more or less densely with such macromolecules that it seems impossible to find the correct solution of our morphological problem.

Fortunately, nature helps us by its innate tendency to repeat certain arrangements indefinitely. This principle of repetition, which is the basis of polymerization, lattice formation, segmentation, and metamerism, renders our task somewhat easier by reducing the indefinite number of possible arrangements so that it is not entirely hopeless to bestow our interest upon the macromolecular structure of cytological objects.

It is my intention to show how far such a morphological analysis on the macromolecular level is possible in cytology. As a botanist I select, by preference, constituents of plant cells, which are familiar to me.

1

The Fine Structure of Starch Grains

Morphology

The starch grains grow by apposition in the interior of the so-called leucoplasts, which are the constituents of the plant cell that polymerize glucose to chain molecules of amylose and amylopectin. As a result of this type of growth the starch grains are layered. In many cases this layering is eccentric (Fig. 1). As early as 100 years ago, Nägeli (1858)

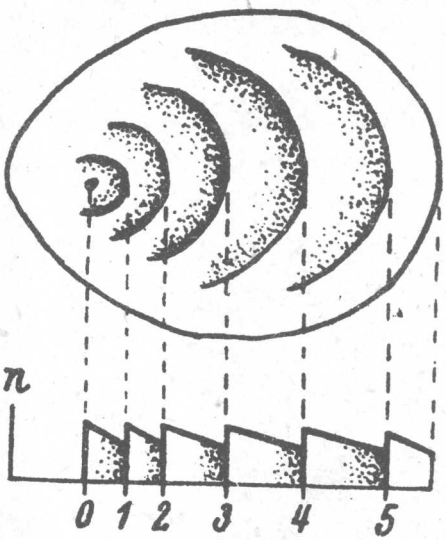


Fig. 1. Layering of the potato starch grain. The density of the outer part of the layers, as judged from their refractive index n, is less than that of the inner part (Frey-Wyssling 1938).

stated in his fundamental book on starch grains that visibility of the layers in the microscope is due to a different hydration of adjacent layers, and by an analysis of the optical density (Frey-Wyssling 1936) it can be shown that the water content rises continuously within a layer in the direction away from the center and falls off abruptly at the layer boundary.

Figure 1 reproduces the inverse course of the refractivity.* Owing to this situation, the layering disappears when the starch grains are dehydrated. This effect is so pronounced that, even in the electron microscope, the grains show no visible structure (Mühlethaler 1955*b*) or only a suggestive layering (Whistler, Byrd, and Thornburg 1955).

In spite of this state of affairs, we know a lot about the submicroscopic structure of starch grains, thanks to the indirect methods of investigation (polarizing microscopy and x-ray analysis). As mentioned, these methods are older than electron microscopy. But they are still useful and, in our special case, even indispensable.

In the polarizing microscope the starch grains display the well-known beautiful "polarization cross." This proves that such a grain is of spherical structure and comparable to a spherocrystal. The larger refractive index of this birefringent spherulite runs radially. Since the polarizability of carbohydrate chain molecules is such that the larger refractive index is directed parallel to the chain axis, the amylose chains cannot run tangentially but must be radially orientated. This accords with the radial cracks that are often produced when starch grains are highly desiccated. But it seems to be in disaccord with the amylose helix devised by Rundle and coworkers (1944), since so flat a pitch of the helix would produce a spherulite with its larger refractive index in the tangential direction if the helices were orientated radially (Frey-Wyssling 1940*a*,*b*). However, the model of Rundle is related to dissolved amylose, in which state the shape of the chain molecules may represent a flatter coil than in the crystal lattice.

Crystallography

This lattice has been studied by x-ray analysis. The big handicap for such an investigation is the spherical structure of the starch grain which does not allow of attributing the diffraction lines on the x-ray diagram to definite directions in the lattice. Kreger (1951) overcame this difficulty by constructing a micro x-ray diffraction camera which enables only a sector of a single starch grain to be irradiated. Of course, the biggest

* Refractivity has been deduced from the displacement of the Becke lines when the microscopic image is brought out of focus. Hess (1955) published photometric measurements of the brightness of successive layers, and from the symmetry of the curves obtained it is concluded that the layers are of uniform, higher or lower, density. However, this method measures light that is intensified or weakened by refraction and diffraction phenomena, and it does not, therefore, allow of estimating the density of the layers, which are all evenly transparent.

starch grains at hand are used for this purpose, such as the giant grains (up to 80 μ in diameter) in the pseudobulb of the orchid *Phajus grandifolius* which sometimes show a peculiar type of lateral apposition growth with almost straight layers (Fig. 2).

As a result, an orthorhombic unit cell is found with the dimensions

$$a : b : c = 9.0 : 10.6 : 15.6 \text{ A.}$$

It contains 3 starch chains with a total of 9 glucose residues and 9 molecules of crystal water. The density of packing is equal to that in crystallized

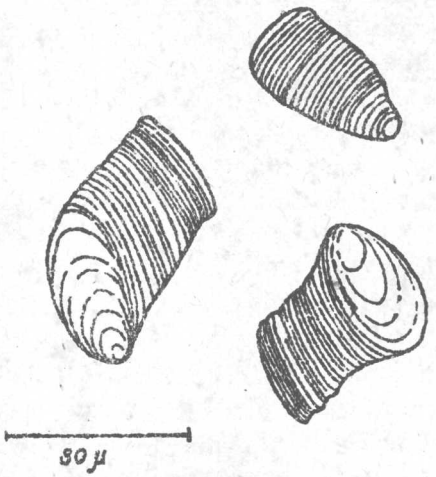


Fig. 2. Starch grains of *Phajus grandifolius* particularly suitable for obtaining a fiber pattern (Kreger 1951; reproduced by permission of Elsevier Publishing Company, Amsterdam).

cellulose, since the density under water of starch grains (1.60–1.63) is similar to that of pure native cellulose (1.59) (Hermans 1949). As the ratio $a : c$ equals $1 : \sqrt{3}$, the symmetry of the lattice is hexagonal. The period 10.6 corresponds to the radial direction of the grain, that is to the chain axis. According to Kreger, the α-glucosidic starch chain forms a threefold helix around this axis.

The starch grains contain only a small amount of amylose; some percentages are: tapioca, 17; rice, 17; banana, 20.5; corn, 21; potato, 22; wheat, 24; sago, 27; lily bulb, 34. In general, it is less than 25 per cent (Bates, French, and Rundle 1943), the rest being amylopectin. Therefore, the question arises how the branched chains of that molecule can be fitted into Kreger's crystal lattice. The difficulty is that the branched

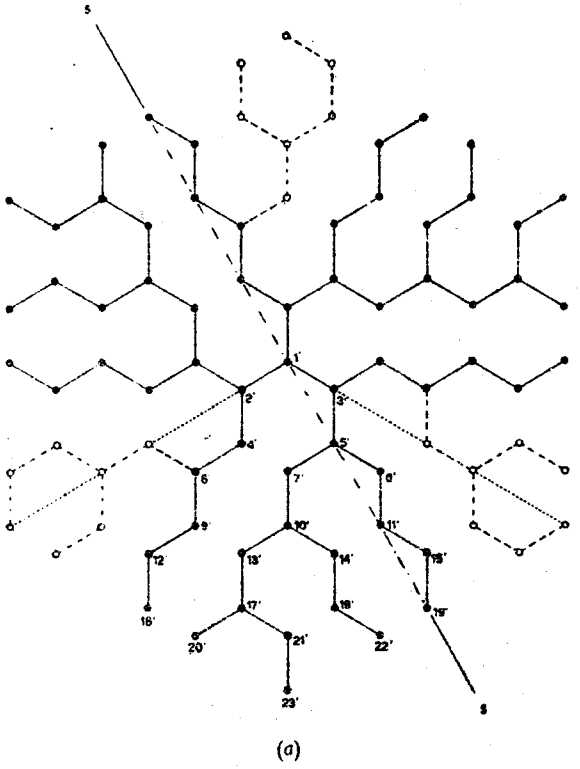
molecules of amylopectin are brushlike, so that there is the problem of how to fill the space completely with such unwieldy particles (Frey-Wyssling 1948*a*).

Amylopectin has a molecular weight between 0.1 and 1 million. This corresponds to a degree of polymerization of about 2000, if we admit a molecular weight of 1/3 million (glucose residue = 162). When this molecule is completely methylated and then hydrolyzed, about 3–5 percent of endgroups (2,3,4,6-tetramethyl glucose) and a similar number of bifurcations (2,3-dimethyl glucose) are found (Meyer 1943, Meyer and Settele 1953). Since the chains are arranged in a hexagonal manner, a bifurcation pattern that fits into the hexagonal system must be found, that is, the twofold symmetry of branching must be combined with the threefold symmetry of the hexagonal system. If the second of the two arrangements proposed by Kreger (1951, p. 421) is chosen, the ramification diagram of Figs. 3*a* and 3*b* can be taken into consideration. A longitudinal section *s–s* of the model is shown in Fig. 3*c*. Such an amylopectin molecule would have 85 endgroups and 84 bifurcations, that is, about 4 percent of 2000. The bifurcations perpendicular to the section *s–s* are indicated by dotted circles. The molecule of amylopectin sketched in Fig. 3 represents a hexagonal prism with a rhombohedral pyramid. This model enables the space to be fitted without gaps or interstices if the crystallites are antiparallel.

The model shows long and short distances between subsequent bifurcations (Fig. 3*b*). The group of glucose residues between two ramifications may be called a member of the amylopectin molecule. The long members are twice as long as the short ones. The model comprises 644 short members in 14 storys, and since the degree of polymerization is 2000, every one contains 3 glucose residues. As their length is 10.6 A, the whole molecule is $14 \times 10.6\ \text{A} = 150\ \text{A}$ long.

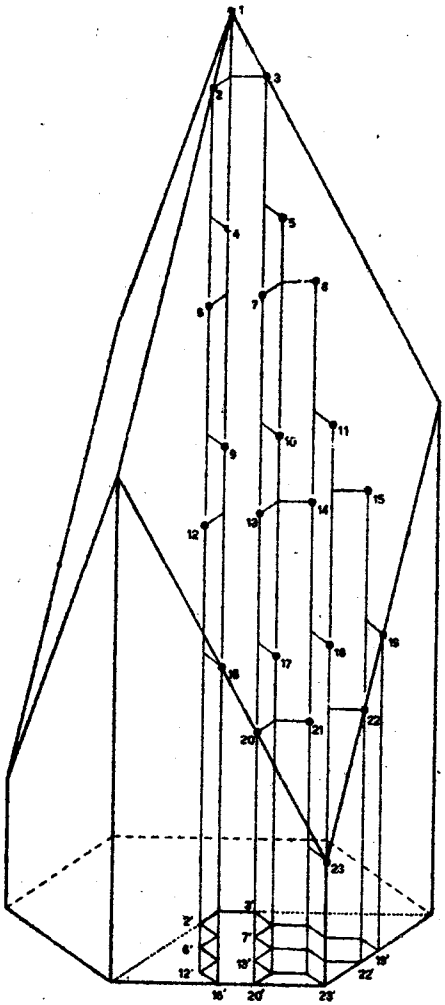
In Fig. 3*b* there are not only bifurcations but also ramifications with three branches. This is the result of a simplified construction. In fact, the chains represented by straight lines are threefold screw axes, so that the branching occurs on slightly shifted levels, as seen in Fig. 3*c*.

The branching of the 4-1-glucosidic main chain is due to 6-1-glucosidic bonds. Since the distance of the parallel chains in the lattice is 6 A, this interval cannot be bridged by such a bond alone, but an additional glucose residue is needed for this purpose (Fig. 4). The whole amylopectin brush has but one open aldehydic group at its tip. This explains the low reducing power of starch.

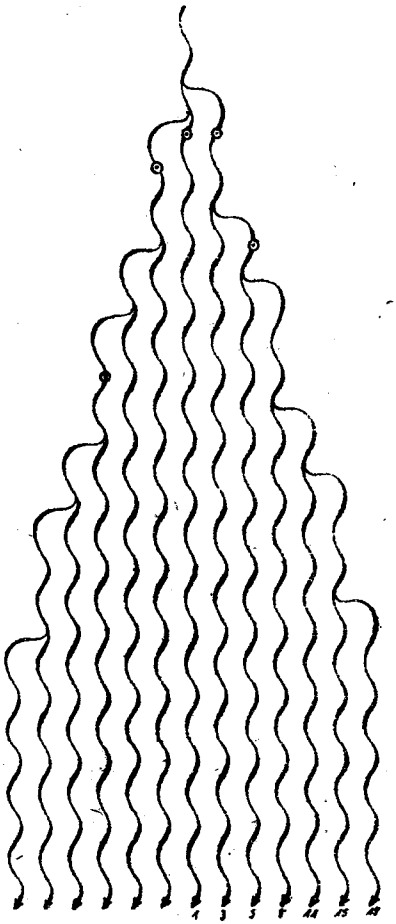


(*a*)

Fig. 3. An attempt to fit amylopectin into the hexagonal lattice of crystallized starch found by Kreger. (*a*) Ground plan showing a hexagonal bifurcation scheme. Each solid circle represents a chain running perpendicular to the drawing plane. Numbers 1′, 2′, . . . indicate the projection of the chains represented in Fig. 3*b*; *s*–*s*, section through the molecule reproduced in Fig. 3*c*. Side length of the hexagons is 6 A. (*b*) View of the molecule of amylopectin, a combination of a rhombohedron and a hexagonal prism permitting close packing. (See Fig. 5*a*,*b*.) (*c*) Longitudinal section through the molecule showing the bifurcations and the helical course of the chains.



(b)



(c)

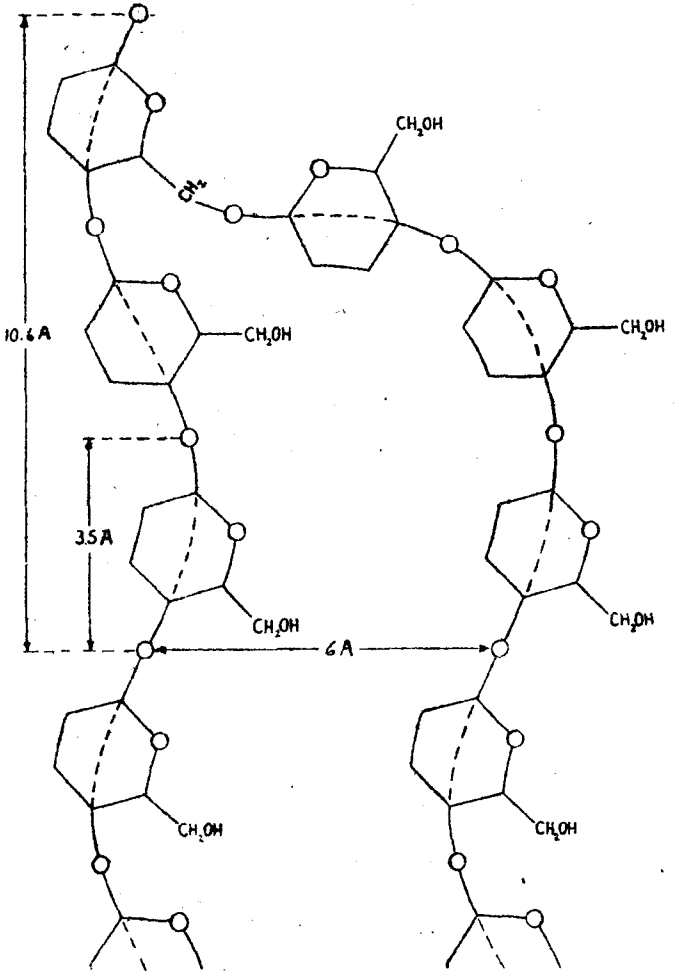


Fig. 4. Possible aspect of the 1–6 bond bifurcation of the helical chains of amylopectin.

If we try to fit these pyramidal molecules into the layer of a starch grain, it is evident that a dense filling of the space according to the high density (1.61) of starch is possible only if the molecules of amylopectin are anti-parallel (Frey-Wyssling 1948*a*). Figure 5*a* shows possible arrangements of this kind.

According to K. H. Meyer (1952), the inner part of the layers of a starch grain consists essentially of amylose and the outer part essentially of amylopectin. On the other hand, Badenhuizen (1938, 1955) demonstrates that there is no such differentiation inside the starch grain. He

claims starch to be a chemically uniform substance and not a mixture of two different compounds. The unbranched amylose molecules are considered as degradation products which are not preformed in the starch

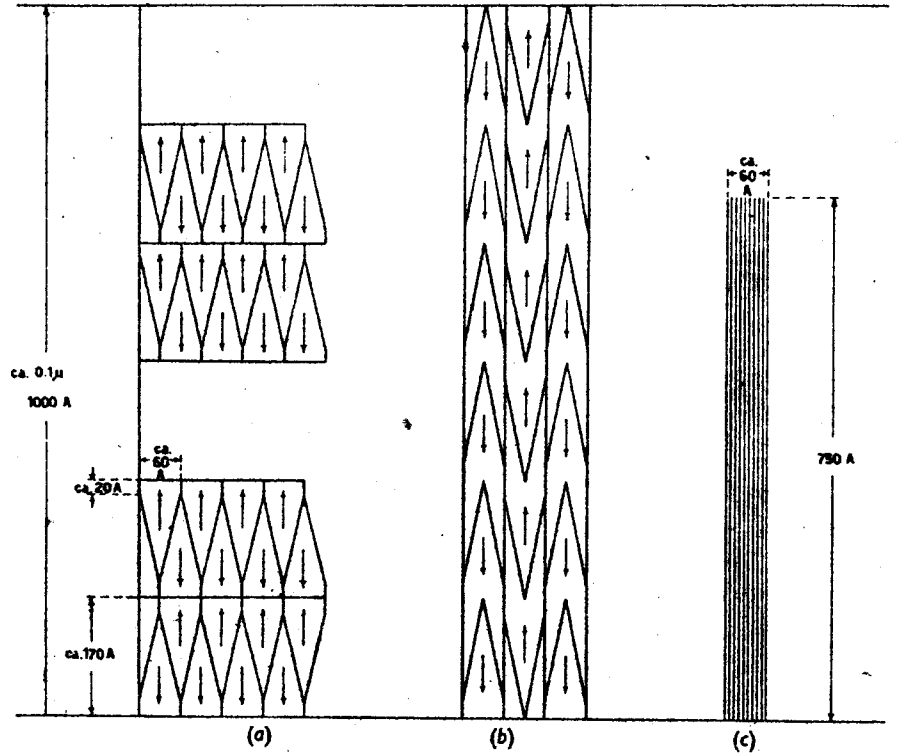


Fig. 5. Possible arrangement of the polar (↓) molecules of amylopectin in the layers of the starch grain. (*a*) Close packing of the shape in Fig. 3*b*. Such a pattern with alternating polarity (↓) does not permit insertion of the much longer chains of amylose (Fig. 5*c*). (*b*) Continuous polarity of antiparallel series of amylopectin molecules. (*c*) Length of amylose chains.

grain. While such a conclusion seems not unlikely from a biological viewpoint, there is the serious difficulty that values from 250 up to 900 are found for the degree of polymerization of amylose (Meyer 1952, Whelan 1955); this gives chain lengths of the order of 1000 A (0.1 μ), while the branched starch molecules of amylopectin, even though ten times as heavy (degree of polymerization, 2000 up to over 10,000) are only some 100 A long. So the origin of the much longer molecules of amylose in liquefied and dissolved starch is obscure, if they are considered as branches of degraded amylopectin.

Meyer and Menzi (1953) suggest mixed crystals of amylopectin and amylose; of course, such a view would seem more likely if molecules of the two types were of similar length, or if the relatively small amount of amylose consisted of shorter chains than the bulk of amylopectin brushes.

The antiparallel arrangement of the amylopectin molecules, as shown in Fig. 5*a*, does not allow of a joint crystallization with the amylose molecules, 5 times as long (Fig. 5*c*), of which the polarity is the same along the whole chain. Mixed crystals are conceivable only if amylopectin molecules are arranged in series as indicated in Fig. 5*b*. In the model according to Fig. 3*b* the base of the molecules has been assumed to be plane. However, if all amylopectin branches are of similar length, the base of the molecule must be hollow to enable the tip of the next molecule to fit into the cavity. In such a model amylose chains of any length can be involved in joint crystallization with amylopectin.

In Fig. 3*a* the points indicated by open circles cannot be placed on the rhombohedral plane, but according to the number of branching steps they ought to be situated in a lower level. If those places are considered to be empty, they can be filled with amylose chains instead of amylopectin branches; then a ratio results of 467 amylopectin members, each with three glucose residues on 63 branches, to 177 amylose members on 21 chains. This means about 75 percent amylopectin and 25 percent amylose, corresponding to the average result of starch analysis. The interrelation of the two components is such that the chemical homogeneity of the starch grain postulated by Badenhuizen (1938) is warranted. At the same time, chemical bonds between amylopectin and amylose are conceivable, so that these two constituents of the dissolved starch do not necessarily need to be individually preformed in the grain.

Indeed, an antiparallel arrangement of the amylopectin molecules is not absolutely necessary in Fig. 5*b*; but as long as no chemical polarity has been found in the layers of the starch grains, polymerization in both possible radial directions seems natural. Of course, the crystallites have no plane faces anywhere, and no level arrangement of the molecules is to be expected. Therefore, the patterns of Figs. 3*b* and 5 must be considered as schematized tentative models of amylopectin designed to show how, based on our actual knowledge of its symmetry, the space in a layer of the starch grain can be densely filled.

The helical structure of the chains and the possibly antiparallel orientation of the molecules in starch must be considered as two general features