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The Past and Present of Starch Chemistry

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Editorial Remark:—On April 14, 1952, our esteemed coworker, Professor K. H. MEYER, died of a very severe illness. He was just able to finish his last paper. He had intended to publish it in *Experientia*, a journal in which he had published frequently. He was always on the best of terms with our editorial staff. Our readers will welcome this survey *in memoriam* to this well-known and respected scientist. H. M.

Introduction

As early as 1811 KIRCHHOFF[2] discovered that grape sugar is produced when starch is treated with acids. Some years later[3] he observed that wheat gluten, or its aqueous extract, converts potato-starch paste into another sugar and he concluded that a starch-sugar transformation is a necessary step in the alcoholic fermentation of amylaceous material. In 1831 LEUCHS[4] noted that saliva brings about the liquefaction of starch, with simultaneous production of a sugar. Two years later PAYEN and PERSOZ[5] obtained from malt a solid amorphous substance which also produced sugar from starch and which they called diastase. These investigations mark the beginning of the structural chemistry of starch; they also mark the birth of a new branch of biochemistry, namely enzymology.

Degradation by amylases, as diastases (i.e. starch splitting enzymes) are now called, has always served as a tool in starch chemistry. The synthesis and degradation of starch play an outstanding role in the biochemistry of plants. In addition, starch is a principal human foodstuff; it is degraded in the body by specific enzymes. Starch is the primary material in the beer and alcohol industries where it is submitted to diastatic breakdown. It is not surprising, therefore, that many botanists, chemists, technologists, and enzymologists have worked on starch and its degradation. By 1930 the number of publications in this field amounted to more than 4000[1].

However, the result of all this research was not satisfactory; it was pointed out in 1930 by the french biochemist SCHOEN[2], that "... in spite of the enormous number of investigations, the study of starch is far from being completed. All investigators agree on this point. It is, in fact, the only point on which there is general agreement in this most controversial aspect of biological chemistry; all other observations are hotly disputed. The origin, constitution, and structure of starch are matter for discussion and there is no unanimity over the way in which it is disaggregated during hydrolysis. Most serious of all, it is not yet known whether or not starch is a single chemical compound."

During recent years, however, most of these questions have been answered. We established in 1940 that most starches contain as principal constituents two polysaccharides, or rather types of polysaccharide, the structures of which are essentially different[3]. They are:

(1) an unbranched polysaccharide, or mixture of unbranched polysaccharides (now known as amylose), in which from 100 to 2000 anhydro-glucose units are joined together by α-1:4 (maltose) bonds to form chains of various lengths (Fig. 1).

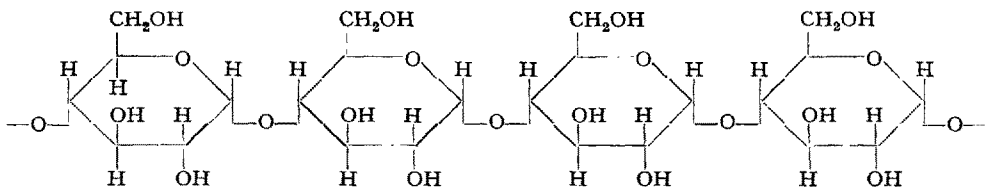

Fig. 1. Amylose.

(2) a highly branched polysaccharide (now termed amylopectin), in which the branches are joined to the

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[2] G. C. S. KIRCHHOF, Mém. Acad. imp. Sci. St. Petersburg *4*, 27 (1811).

[3] G. C. S. KIRCHHOF, Schweigger's J. Chem. Phys., Nuremberg *14*, 389 (1815).

[4] E. LEUCHS, Poggendorf's Ann. Phys. Chem. *22*, 623 (1831).

[5] A. PAYEN and J. PERSOZ, Ann. Chim. Phys. *53*, 73 (1833).

[1] R. P. WALTON, *A Comprehensive Survey of Starch Chemistry* (Chemical Catalog Co., New York, 1928).

[2] M. SCHOEN, Bul. Soc. Chim. biol. *12*, 1033 (1930).

[3] K. H. MEYER, Naturwissenschaften *28*, 397 (1940); Advan. Coll. Sci. *1*, 143 (1942).

main chains by α-1:6 (isomaltose) bonds the main chains and branches being structurally the same as amylose (Figs. 2 and 3).

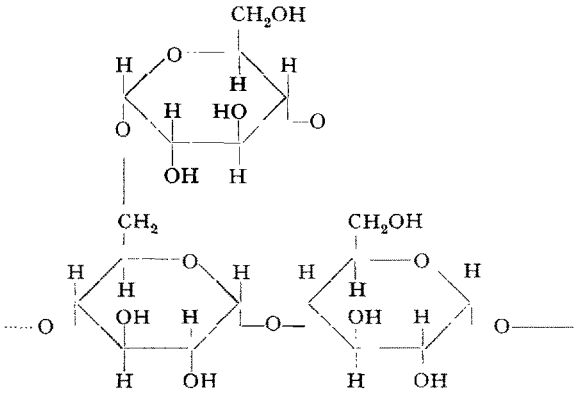

Fig. 2.

In contrast, the starches from corn, rice, and millet, that are stained purple by iodine and are called glutinous or waxy starches, are homogeneous; they contain only the branched polysaccharide[1]. From 1946 on, the isolation of various pure crystalline amylases has brought about remarkable progress in our understanding of the enzymology of starch breakdown. Furthermore we have to-day a well founded insight into the submicroscopic structure of the starch granule.

It seems therefore that research in this field has now advanced to a point where it would be of great interest to consider the evolution of starch chemistry from its very origin. Before surveying our present knowledge of the subject, we shall recapitulate some of the older results of botanists, colloid-scientists, organic chemists, and enzymologists, treating them separately. Such a procedure is justifiable because, until recent times, research on starch in these different branches of science has not been coordinated, and this partly accounts for the astounding fact that the structure of starch was established much later than those of numerous alkaloids, vitamins, and hormones whose constitutions were determined soon after their discovery.

(1) *The Botanist's Contribution.*–Many leading botanists have worked with starches. Their extensive microscopical studies showed that the granules from various plants are so characteristically differentiated in size and shape that one can determine the origin of a starch by microscopy[2]. Nevertheless, all types of starch granule are composed of layers and all exhibit a black cross when viewed in polarised light. This cross is typical of spherulites which are spherical aggregates of radially oriented needle-like crystals. The individual crystals are not, however, visible under the microscope; hence NAEGELI[1] concluded that they are submicroscopic and he called them "crystalline micelles". MEYER[2], who continued this work, thought that the crystallites might be branched and called them "trichite", but only recently has a clear understanding been reached of their nature and function (cf. section 7).

Botanists have also contributed to the chemistry of starch. As early as 1712 LEEUWENHOEK[3] supposed the granules to possess a resistant shell different from the water-soluble interior. NAEGELI confirmed the older observation of PAYEN and PERSOZ that malt degrades only part of the granule, the "shell" remaining intact. Hence, and also because the malt-treated shells did not give a blue colour with iodine, NAEGELI concluded that the shells are chemically different from the contents and are made of a cellulose-like substance (amylocellulose). MOHL[4], MUSCULUS[5], MEYER and DE VRIES[6] showed, however, that the shells can be dissolved in alkali and not only remain in solution after neutralisation but also stain with iodine. Subsequently the polysaccharide is slowly deposited from solution. It is now realised that the shell is composed of a difficultly soluble aggregate comprising a mixture of high molecular-weight linear and branched polysaccharides which may be brought into super-saturated solution by dissolving in alkali and neutralising. At the time the above mentioned botanists assumed that there are various starch components present in the form of different aggregates of the same basic substance. This was termed "amylose" by MEYER, the insoluble aggregate being distinguished as α- and the soluble form as β-amylose. FREY-WYSSLING[7] and ALSBERG[8] also thought that the fractions "amylose" and "amylopectin", which are to be discussed later and are synonymous with MEYER's β- and α-amylose, might be fractions of different molecular weight of the same basic substance. HANES[9], whose important work on the phosphorolysis of starch will be referred to later, also advocated this conception, as did BADENHUIZEN[10] who published in 1938 under the characteristic title, *The Starch Granule as a Chemically Homogeneous Entity.*

(2) *The Colloid Chemist's Contribution.*—It was found by technologists[11] as well as botanists that under normal

[1] K. H. MEYER and M. FULD, Helv. chim. Acta *24*, 1404 (1941) (glutinous rice). – K. H. MEYER and M. HEINRICH, Helv. chim. Acta *25*, 1639 (1942) (waxy maize).

[2] E. T. REICHERT, *The Differentiation and Specificity of Starches in Relation to Genera, Species, etc.*, Washington, Carnegie Publ. No. 173 (1913).

[1] C. V. NAEGELI, *Die Stärkekörner* (Pflanzenphysiologische Untersuchungen, Zürich, 1858).

[2] A. MEYER, *Untersuchungen über die Stärkekörner* (Fischer, Jena, 1895).

[3] A. LEEUWENHOEK, Epistolae *26* (1719).

[4] H. MOHL, Bot. Ztg. *17*, 225 (1859).

[5] F. MUSCULUS, Bot. Ztg. *37*, 345 (1879).

[6] H. DE VRIES, Rec. Trav. chim. Pays-Bas *4*, 187 (1885).

[7] A. FREY-WYSSLING, *Submikroskopische Morphologie des Protoplasmas* (Berlin, 1938), p. 277.

[8] C. ALSBERG, Plant Physiol. *13*, 295 (1938).

[9] S. HANES, New Phytologist *36*, 101, 189 (1937).

[10] N. P. BADENHUIZEN, Rec. Trav. bot. nederl. *35*, 561 (1938).

[11] O'SULLIVAN, J. Chem. Soc. *35*, 770 (1879). – H. T. BROWN and J. HERON, J. Chem. Soc. (London) *35*, 596 (1879).

conditions starch pastes yield only about 80% of fermentable sugars on degradation. MAQUENNE and ROUX[1] concluded that starch contains 20% of a resistant substance that remains unchanged after fermentation of the sugars, does not stain with iodine, and is akin to the pectins and plant mucilages. This substance they called "amylopectin". The part of the starch that is degraded to maltose and stains iodine was termed "amylose". These views were widely criticised, and vigorously condemned by SCHOEN[2]. It appears that MAQUENNE and ROUX did not even consider the possibility that a polysaccharide might be only partially attacked;—but this is in fact the case! We now know that both the common heterogeneous starches and the homogeneous glutinous types are rapidly degraded only to 80%, and that the residue consists of oligosaccharides, tetraoses, and trioses which are unfermentable and difficult to hydrolyse. It is incorrect, therefore, to ascribe the discovery of the heterogeneous nature of starch to MAQUENNE and ROUX. Nevertheless we see how the expressions "amylose" and "amylopectin" originated, although they have been generally employed in other senses.

Most authors have identified the resistant material of the shell as "amylopectin" and the readily soluble contents as "amylose". Many methods have been suggested for the separation of the two substances: treatment of starch with concentrated caustic soda and precipitation of the amylopectin with alcohol[3]; extraction of amylose with warm water[4]; precipitation of amylopectin from dilute pastes with calcium chloride[5]; freezing and thawing of pastes, when the amylopectin remains as a fibrous mass[6]; and separation of "amylopectin" from pastes by centrifuging[7]. Several authors isolated the "amylopectin" alone by treatment of the starch with diastase. They seem to have given as little attention as MAQUENNE and ROUX to the possibility that amylopectin itself might be attacked. Constitutional differences between the fractions have been repeatedly sought; but neither HIRST[8] nor KARRER[7] could find any, and HAWORTH[9] subsequently defined degraded starch as "amylose". We have recently established that the "amylopectins" obtained by the above methods are high molecular-weight mixtures of both linear and branched starch polymers while the "amyloses" are simply mixtures of lower molecular-weight.

[1] L. MAQUENNE and E. ROUX, Ann. Chim. Phys. *9*, 179 (1906).
[2] M. SCHOEN, Bul. Soc. Chim. Biol. *12*, 1033 (1930).
[3] Z. GATIN-GRUZEWSKA, C. r. Acad. Sci. *146*, 540 (1908); Z. Physiol. Path. Gen. *14*, 7 (1912). – F. BOTTAZI and C. VICTOROV, Real Acad. Linc. Chim. Phys. (Roma) *19*, (5) 7 (1910).
[4] C. TANRET, C. r. Acad. Sci. *158*, 1353 (1914).
[5] J. T. L. ZWIKKER, Rec. Trav. Bot. neder. *18*, 78 (1921).
[6] A. R. LING and D. R. NANJI, J. Chem. Soc. (London) *123*, 2666 (1923).
[7] P. KARRER and P. KRAUS, Helv. chim. Acta *12*, 1144 (1929).
[8] E. L. HIRST, M. PLANT, and M. D. WILKINSON, J. Chem. Soc. *1932*, 2375.
[9] D. K. BAIRD, W. N. HAWORTH, and E. L. HIRST, J. Chem. Soc. *1935*, 1201.

The electro-decantation of starch leads to other results. This method is due to SAMEC[1], who has devoted his life to the investigation of starch. At the anode "amylopectin" is deposited, this we have found to contain the greater part of the branched polysaccharide. In solution there remains what SAMEC called "amylo-amylose" and which appears to be the unbranched carbohydrate. SAMEC himself established that amylo-amylose stains an intense blue colour with iodine and is wholly degraded to maltose by barley amylase (β-amylase) while the "amylopectin" stains violet red and is degraded only to the extent of 60%[2]. On the basis of these important, but little noticed, results SAMEL concluded that there is a constitutional difference between the two polysaccharides, but left the question of their nature to the organic chemists, observing that: "... the answer will not be long hidden from modern organic research"[3].

Independently of SAMEC we were studying the systematic fractionation of starch from 1935 onwards. We found that on careful treatment of the granules with water at, or about, 60°C a polysaccharide goes into solution. This substance is differentiated from the principal, branched, polysaccharide by its intense blue iodine compound and also by the fact that its triacetate can form strong threads, comparable with those from cellulose[4]. We decided therefore on an unbranched structure similar to that of cellulose and we were shortly able to confirm this structure by chemical methods[5]. Our product was completely degraded by β-amylase in the same manner as SAMEC's amylo-amylose. Thus the question mentioned above was answered even before it had been posed.

Neither SAMEC's nor our fractionation method is perfect; with both, one is left with a mixture of branched and linear polysaccharides. It is not therefore surprising that STAMBERG[6] found on electrodialysis only 20% "amylopectin" and 80% "amylose" in wheat starch although the branched polysaccharide does in fact comprise 80% of the whole. Furthermore, FREUDENBERG[7] could find no constitutional difference between the "amylo-amylose" and "amylopectin" prepared by SAMEC's method, and earlier workers had no success with the method used in our laboratory. The best method of fractionation is that discovered by SCHOCH[8]. Addition of butanol to a pressure-cooked starch paste causes a polysaccharide-alcohol complex (SCHOCH's "A" fraction) to separate, and this poly-

[1] M. SAMEC, *Kolloidchemie der Stärke* (Verlag Th. Steinkopf, Leipzig, 1927).
[2] M. SAMEC and E. WALDSCHMIDT-LEITZ, Z. Physiol. Chem. *203*, 16 (1931).
[3] M. SAMEC, Ber. dtsch. chem. Ges. [A] *73*, 85 (1940).
[4] K. H. MEYER, C. r. Soc. Phys. Hist. Nat. Genève *57*, 19 (1940).
[5] K. H. MEYER, Naturwissenschaften *28*, 397 (1940).
[6] E. O. STAMBERG, Cereal Chem. *17*, 342 (1941).
[7] K. FREUDENBERG and H. BOPPEL, Ber. dtsch. chem. Ges. *71*, 2505 (1938).
[8] T. J. SCHOCH, Cereal Chem. *18*, 121 (1941).

saccharide was later found to be the pure linear component[1]. Substantially pure branched component remains in solution.

Colloid chemists have made intensive investigations on the problem of the structure of "pastes"—those remarkable viscous systems which are formed on heating starch in water. After long investigation SAMEC[2] came to believe that the properties of starch pastes and solutions are essentially determined by the small quantities of non-saccharide impurities. The phosphorus content of the paste-forming amylopectin appeared to him of especial importance since the non paste-forming "amylo-amylose" is free from phosphorus. MALFITANO[3] also considered the phosphorus content of first importance. According to him, starch is an aggregate of individual complexes each of which is centred around a phosphorus atom—almost as in the heteropolyacids, such as phosphotungstic acid. However, POSTERNAK[4] has now shown that in cereal starches the phosphorus is not combined with the polysaccharide but exists as phosphatide in the accompanying fats. Since both the original and de-fatted starches yield identical pastes, both SAMEC's and MALFITANO's ideas must be incorrect.

A "colloid chemical" theory which also completely ignored the chemical constitution of starch was proposed by VON DER HOEVE, BUNGENBERG DE JONG, and KRUYT[5]. According to these authors, the grain is built up of negatively charged micelles that are held together at various places by means of a water layer in which the water is in an "abnormal state". The force exerted by the swelling granules was accounted for by capillary-electric repulsive forces between the likecharged micelles. However, granules from which the ionised phosphatide groups have been removed swell quite as well as native granules, and hence electrical forces can play no role in swelling phenomena.

We shall see later (Section 8) that the chemical constitution of the polysaccharide as well as the submicroscopic structure of the granules must be considered in any attempt to elucidate the mechanism of paste formation.

(3) *The Organic Chemist's Contribution.*—As early as 1814 KIRCHHOFF[6] discovered that the sugar obtained from starch by the action of malt can be crystallized from alcohol and is different from the glucose obtained on acid-hydrolysis. DUBRUNFAUT[7] again isolated the sugar in 1847 and called it "maltose". This work was also overlooked and the re-discovery is due to O'SULLIVAN[8]. The constitutions of both sugars have been unequivocally established by the outstanding work of HAWORTH[1].

Since maltose, produced by the degradation of starch, is composed of two glucose residues connected by an α-1:4 glucosidic link, it was generally believed up to 1920 that there exists in starch a high molecular-weight polysaccharide whose glucose residues are joined solely by maltose-type bonds. This viewpoint was advocated for example in the wellknown text-book of V. MEYER and P. JACOBSON[2].

In 1920 commenced a strange phase of the chemistry of the natural polymers–rubber, cellulose, silk, and starch. Molecular-weight determinations by freezing-point depression gave strikingly low values, and hence originated the theory that these substances are composed of low molecular-weight units that associate to form larger entities through special bonding forces. As primary unit of the various types of starch, including so-called soluble starch, the following were suggested: an anhydro-trisaccharide[3]; a tri-hexosan for the insoluble outer layer and a di-hexosan for the soluble contents[4]; a maltose anhydride[5]; a cyclic anhydride of malto-triose[6]; and a cyclic hexaose anhydride[7]. In all cases it was further asserted that there are no free aldehyde groups in starch. This however is to be attributed to the shortcomings of the analytical methods. Hence the discovery of a method that could detect very small reducing powers represented a big step forward, and it was in fact found that all starches exhibit reducing power[8].

By the following method HAWORTH[9] arrived at the concept of a "molecule" of 20 glucose residues. All free hydroxyl groups were first methylated and the product acid-hydrolyzed, the resulting sugar mixture was then analyzed. The mixture yielded 5% tetra-methylglucose which could only have originated from the non-aldehydic end groups. There is therefore one end-unit for each twenty glucose units; "... this corresponds to a chain which is not endless or to a molecular weight of not more than 4000". These fragments of about 20 residues were also known as repeating units; they were supposed to aggregate through bonds of unspecified type to form the various kinds of starch.

According to HAWORTH, "amylopectin" can be disaggregated to "amylose" and then be re-formed on standing[10].

[1] K. H. MEYER *et al.*, J. Phys. Coll. Chem. *53*, 319 (1949).
[2] M. SAMEC and M. BLINC, Koll. Beih. *47*, 317 (1938).
[3] G. MALFITANO, Koll.-Z. *46*, 3 (1928).
[4] T. POSTERNAK, Helv. chim. Acta *18*, 1351 (1935).
[5] A. V. D. HOEVE, H. G. B. DE JONG, and H. R. KRUYT, Koll. Beih. *39*, 105 (1934).
[6] G. C. S. KIRCHHOFF, Schweigger's J. F. Chem. Phys. Nuremberg *14*, 389 (1815).
[7] A. DUBRUNFAUT, Ann. Chim. *21*, 178 (1847).
[8] C. O'SULLIVAN, J. Chem. Soc. *25*, 579 (1872).
[1] W. N. HAWORTH, Helv. chim. Acta *11*, 534 (1928).
[2] V. MEYER and P. JACOBSON, *Lehrbuch der Organischen Chemie*, Vol. I, part 2, 2nd Ed. (Verlagde Gruyter, Berlin, 1913), p. 1024 et seq.
[3] A. PICTET *et al.*, Helv. chim. Acta *7*, 934 (1924); *12*, 700 (1929); *10*, 276 (1927).
[4] H. PRINGSHEIM, *Die Polysaccharide*, 3rd Ed. (Berlin, 1931).
[5] P. KARRER, *Polymere Kohlenhydrate* (Leipzig, 1925).
[6] J. C. IRVINE and J. MACDONALD, J. Chem. Soc. *1926*, 1502.
[7] A. R. LING and D. R. NARJI, J. Chem. Soc. *123*, 2666 (1923); *127*, 629 (1925).
[8] W. A. RICHARDSON, R. S. HIGGINBOTHAM, and F. D. FARROW, J. Text. Inst. *37*, 131 (1936).
[9] W. N. HAWORTH, Nature *129*, 365 (1932).
[10] W. N. HAWORTH, Chem. and Ind. *53*, 1059 (1934).

Despite these association theories KUHN[1] and FREUDENBERG[2], as well as MEYER[3], continued to advocate the high molecular-weight nature of starch. It was shown that practically all the bonds in starch are hydrolyzed by acids at the same rate and must be glucosidic. MEYER also observed that an unbranched chain of maltose residues could never account for the properties of starch, whose molecules must be highly branched or cross-linked.

The theory of the branched structure of starch was taken up by STAUDINGER[4]. HAWORTH's end-groups became the ends of branches (not of molecules), an interpretation with which HAWORTH and his school concurred. STAUDINGER even proposed a special constitution viz. a main chain to which long branches are attached at intervals. Today this form is outdated, and the same can be said of HAWORTH's "laminated" structure, both ideas being in contradiction with the evidence from enzymic degradation.

Difficulties have also arisen because many chemists have treated "starch" as a homogeneous chemical substance, and occasionally still do, in disregard of other work. Such a conception is misleading. Starch granules are individual systems of biological origin; their properties vary with the species of plant, the organ in which they are found, their age, and their pretreatment. Many errors must be attributed to the fact that unfractionated starch or commercial degraded "soluble" starches have been used for structural research work.

In the earliest chemical investigations the question of heterogeneity was not raised. Subsequently KARRER[5], HIRST[6], and FREUDENBERG[7] were among the first to investigate the fractions, "amylose" and "amylopectin", obtained from pastes by centrifuging, electro-decantation, and freezing. They found no constitutional variation, and hence their "fractions" must now be considered to have been mixtures of the linear and branched polysaccharides. FREUDENBERG, like HAWORTH, concluded from his experiments that "amylose" and "amylopectin" differ only in their molecular-weight. Right until 1939 HASSID[8], HAWORTH[9], and HIRST[10] were emphasizing the constitutional homogeneity of what we now know to be the heterogeneous composite starches of Canna, wheat, horse chestnut, and rice. No difference was observed by these authors between the really homogeneous starch from maize and the common heterogeneous starches although quite different colours are given by these substances with iodine (respectively, purple and blue).

REICH and DAMANSKI[1] also defended a theory of homogeneity which was based on a primary substance containing only two free acetylable hydroxyl groups. "Amylose", containing three free hydroxyls, is obtained on hydrolysis. It can easily be shown that these authors acetylated imperfectly in a heterogeneous system, so that their conclusions are unsound. In their work on the periodate oxidation of starch, JACKSON and HUDSON[2] also treated it as a homogeneous substance.

In connection with our fractionation experiments by aqueous extraction of swollen granules we examined the soluble fraction and found it to contain 0·4% non-reducing end groups—one per 250 glucose units. Since the osmotically determined D.P. (degree of polymerization) was also 250 it follows that the molecules of this fraction are unbranched. Nevertheless, the main component of the starch consists of a highly branched polysaccharide of high molecular-weight as is proven by its high yield of tetra- and di-methyl glucoses on methylation and hence its heterogeneity was proven beyond doubt[3]. To end the prevailing confusion in the literature we proposed to re-define the old terms "amylose" and "amylopectin" in the following manner: the unbranched constituent would be called amylose, and the branched constituent, amylopectin.

This proposal was accepted by the Nomenclature Committee of the International Union of Chemistry.

(4) *The Enzymologist's Contribution.*—The story of the chemistry of the amylases, as are now denoted the starch-destroying diastatic enzymes, is not less eventful than that of starch. Early investigators soon established that sugars and dextrins are produced during liquefaction of starch by malt extract. KIRCHHOFF knew in 1815 that malt sugar is different from glucose[4], though this was twice forgotten and twice re-discovered—by DUBRUNFAUT[5] in 1847 and O'SULLIVAN[6] in 1872. The latter also established that, for equal extents of hydrolysis, the rotatory power of the starch solution falls much more at lower temperatures than at higher.

SCHWARZER[7] demonstrated that whereas the maltose producing capacity of aqueous malt extract is greatly diminished on heating, the dextrinizing power remains constant. Hence it was deduced by MÄRCKER[8] in 1877 that malt extract contains two enzymes. This was

[1] R. KUHN, Ann. Chem. *443*, 1 (1925).
[2] K. FREUDENBERG *et al.*, Ber. dtsch. chem. Ges. *63*, 1510 (1930).
[3] K. H. MEYER, H. HOPF, and H. MARK, Ber. dtsch. chem. Ges. *62*, 1103 (1929).
[4] H. STAUDINGER and E. HUSEMANN, Ann. Chem. *527*, 195 (1937).
[5] P. KARRER and E. v. KRAUSS, Helv. chim. Acta *12*, 1144 (1929).
[6] E. L. HIRST, M. PLANT, and M. D. WILKINSON, J. Chem. Soc. *1932*, 2375.
[7] K. FREUDENBERG and H. BOPPEL, Ber. dtsch. chem. Ges. *71*, 2505 (1938).
[8] W. Z. HASSID and W. H. DORE, J. Amer. Chem. Soc. *59*, 1503 (1937).
[9] W. N. HAWORTH, E. L. HIRST, and M. P. WOOLGAR, J. Chem. Soc. 177 (1935).
[10] E. L. HIRST and J. T. YOUNG, J. Chem. Soc. *1939*, 951, 1471.

[1] W. S. REICH and A. F. DAMANSKI, Bl. Soc. Chim. Biol. *19*, 158, 357 (1937).
[2] E. L. JACKSON and C. S. HUDSON, J. Amer. Chem. Soc. *59*, 2049 (1937).
[3] K. H. MEYER, Naturwissenschaften *28*, 397 (1940); Adv. Coll. Sci. *1*, 143 (1943).
[4] G. C. S. KIRCHHOFF, Schweigger's J. Chem. Phys. *14*, 389 (1815).
[5] A. DUBRUNFAUT, Ann. Chim. Phys. *21*, 178 (1847).
[6] C. O'SULLIVAN, J. Chem. Soc. *25*, 579 (1872); *30*, 125 (1876).
[7] A. SCHWARZER, J. prakt. Chemie *109*, 212 (1870).
[8] M. MÄRCKER, Ber. dtsch. chem. Ges. *10*, 2234 (1877).

proven by WIJSMAN[1] in 1889 by a striking and original demonstration in which he separated the two enzymes by their different rates of diffusion. A drop of malt extract was placed on a starch-containing gelatin plate which was sprayed after an interval with an iodine-potassium iodide solution. Where the extract had been placed there was now a colourless spot surrounded by a red-violet ring; the former indicated the presence of "dextrinogen amylase", the latter the "saccharifying amylase". Outside this ring the plate was stained blue because the starch was unchanged.

The rapidly diffusing enzyme changes starch into maltose and a limit dextrin that stains red-violet with iodine; this dextrin was also known as erythrogranulose. Dextrins giving no colour with iodine are produced by the slower diffusing enzyme. This work lays unnoticed for many years and it was not until much later that the remarkable behaviour of the optical rotation during degradation and the reaction mechanisms of the two kinds of enzymic action were clarified. The malt dextrinizing amylase and all animal amylases liberate maltose in the upward rotating α-form, the saccharifying amylase liberates it in the β-form. Consequently the two classes of amylase are referred to as α- and β-amylases[2]. The α-amylase acts at random so that the polysaccharide is split into large fragments. The β-amylase acts only from the non-reducing chain end, splitting off one maltose unit after another[3] (Fig. 4).

SAMEC and WALDSCHMIDT-LEITZ[4] found that SAMEC's "amyloamylose" is quantitatively degraded to maltose by β-amylase. The principal component of starch, however, is changed into a high molecular weight limit dextrin, and MYRBÄCK[5] and HANES put forward the idea that in this case the enzyme is hindered from acting further by foreign groups such as phosphate. We now know that the hindrances are in fact formed by the branch points.

Degradation by α-amylase is more complicated than that by β-amylase and the mechanism was only recently explained. A correlation between dextrinizing action and liquefying power was recognised quite early. Originally however no reducing groups could be detected in the first stages of liquefaction and in consequence a special liquefying or depolymerizing enzyme, without hydrolyzing power, was postulated. It is now realised that lack of a sensitive analytical method led to this hypothesis and that liquefaction is really due to hydrolysis of chain linkages[6]. The belief[7] that an amylophosphatase is necessary for liquefaction is also incorrect since this action occurs with phosphorus-free starch.

As we have seen, the amylolytic breakdown of starch was often used in investigating its structure. Botanists, e.g. NAEGELI, colloid chemists, e.g. MAQUENNE, and enzymologists, e.g. LING, have regarded the portion consumed by amylases as an individual compound, "amylose". Thus they ignored the fact, familiar to organic chemists, that many substances are only partially degraded, and that really the partial degradation is the most important method of structure investigation. LING[1], after many years work on the structure of starch using amylases, arrived at conclusions which are now seen to be untenable. He thought that starch consists of 70% amylose (i.e. everything that yields maltose), 20% amylopectin, which is "depolymerized" to a cyclic hexaose by amylases, and 10% amylohemicellulose which he took to be a Ca-Mg salt of a phosphate-containing hexaose. From his description this last can be nothing other than an impure linear constituent of high D.P. in an insoluble crystalline form.

The discovery of the difference between α- and β-amylases has also led to incorrect deductions that have complicated the already difficult problem of starch structure. KLINKENBERG[2], under the direction of WENT, employed α- and β-amylases to analyse the constituents of starch. He thought to have proved that it consists of two stereo-isomers that can mutarotate. At equilibrium they are found in proportion of 36% α- to 64% β-form. When, however, it is realised that the change α-bond in starch to β-configuration of the maltose reducing group occurs at the moment of hydrolysis by β-amylase, in a kind of Walden Inversion, the hypothesis quoted above is seen to be without foundation. The same observation applies to the hypothesis of BLOM, BAK, and BRAAE[3] which supposes the starch molecule to comprise 30 glucose residues linked by 4 kinds of bond that are distinguished by their reactions with pure α-, pure β-, mixed α- and β-amylases, and with crude malt amylase.

A notable contribution to our knowledge of starch polysaccharide was MYRBÄCK's discovery[4] that branched oligosaccharides containing α–1:6′ bonds occur in the low molecular-weight α-dextrins. In our investigations we degraded the pure polysaccharide constituents with pure β-amylase. The linear fraction was completely degraded, so confirming SAMEC's finding for "amyloamylose". The branched fraction, however, was only degraded to about 65% leaving a β-dextrin that contained all the branch points[5]; these evidently con-

[1] H. P. WIJSMAN, Diss. Amsterdam 1889, Rec. Trav. Chim. Pays-Bas *9*, 1 (1890).
[2] R. KUHN, Ann. Chem. *443*, 1 (1925).
[3] E. OHLSSON, Z. physiol. Chem. *189*, 17 (1930).
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[2] G. A. v. KLINKENBERG, Z. physiol. Chem. *212*, 173 (1932).
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[4] A. MYRBÄCK and K. AHLBORG, Biochem. Z. *307*, 69 (1940).
[5] K. H. MEYER and P. BERNFELD, Helv. chim. Acta *23*, 875 (1940).

stitute the hindrances to the enzyme. Since 65% of the fraction was converted to maltose it follows that 70% of the glucose units are in the branches of the molecule. We also found that under the action of a glucosidase from yeast autolysate the α–1:6′ bonds were scinded and that then the β-amylase further degraded the dextrin. These facts can only be explained by a highly ramified structure such as that shown in Figure 3*a*. This structure has been confirmed by CORI[1]; other branched structures such as those of HAWORTH[2] (3*b*) and of STAUDINGER[3] are in contradiction with the facts.

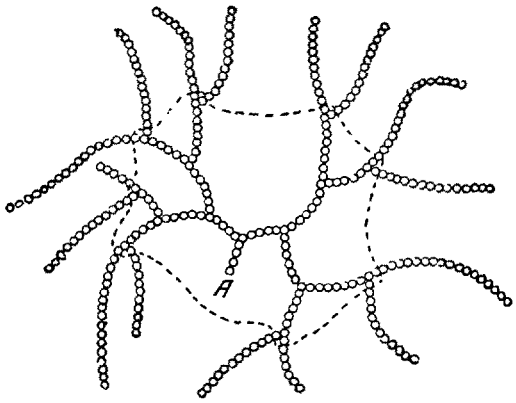

Fig. 3*a*.–Structure of amylopectin proposed by MEYER and BERNFELD. o o o, glucose residues; *A*, reducing group; dotted line, limit of degradation by β-amylase.

As far as the individual products of α-amylase degradation are concerned, starch was treated as a homogeneous substance in almost all the early work. A complete understanding of amylolytic action can however only be attained through investigation of the purified linear and branched component polysaccharides. Attention must also be given to the enzyme because impure samples may contain activators or inhibitors.

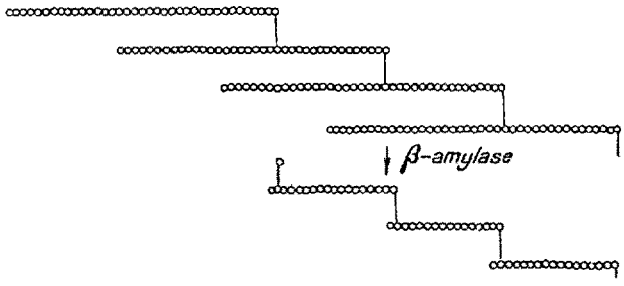

Fig. 3*b*.–Formulae of amylopectin (above) and limit dextrin (below) proposed by HAWORTH.

Many attempts have been made to purify the amylases. The extensive researches of EULER, WILLSTÄTTER, SHERMAN, and HOLMBERGH are noteworthy. While the majority of enzymologists considered enzymes to be proteins, WILLSTÄTTER and WALDSCHMIDT-LEITZ[1] thought that they are nitrogen-free substances belonging to an unknown class of compound. This view can no longer be sustained because, beginning with the β-amylase of sweet potato in 1946, many amylases have been obtained in pure crystalline form and shown to be proteins. We shall refer later to these researches which placed the enzymology of the amylases on a new footing.

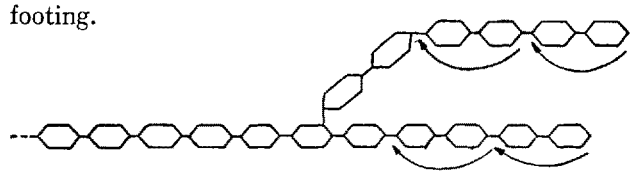

Fig. 4.–Degradation of amylopectin with β-amylase.

(5) *The Starch Polysaccharides.*—Amylose, by which we shall now understand the unbranched polysaccharide, and amylopectin, the branched component, are the principal constituents of starch. Cereal starches contain small quantities of lipids and phosphatides while root and tuber starches are completely free from these impurities; their amylopectins contain small quantities of phosphorus bound to the primary-hydroxyl as ester[2]. All the side branches are attached to the primary-hydroxyl. Branch points other than 1:6′ can be shown to be absent since exhaustive periodate oxidation leaves no unchanged glucose[3]. Hence all units must carry two neighbouring free hydroxyl groups (Fig. 5).

Our later work shows that the granules of common starches contain both low (D.P. 50 → 200) and high (D.P. 200 → 1500) molecular-weight amylose; we refer to them as A_1 and A_2 respectively. They appear to exist in different layers of the granule and fulfil different biological functions. The amylopectin of most starches (B_2) is homogeneous and of high D.P.[4]. However the amylose-free "waxy" starches contain a low molecular-weight amylopectin (B_1) which replaces amylose A_1[5]. Only in tapioca do A_1 and B_1 co-exist[6].

A number of new methods have been discovered for the analysis and characterisation of these polysaccharides. A_1 and B_1 go into solution when the granules are treated with warm water. When a prepared starch solution is saturated with butanol the A_1 and A_2 fraction precipitate as a microcrystalline complex with butanol[7]; any remaining amylose can be removed in combination with stearic acid[4]. Amylose forms a com-

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[2] T. POSTERNAK, Helv. chim. Acta *18*, 1351 (1935); J. Biol. Chem. *188*, 145 (1951).
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[4] K. H. MEYER and G. C. GIBBONS, Helv. chim. Acta *33*, 210, 213 (1950).
[5] K. H. MEYER and P. HEINRICH, Helv. chim. Acta *25*, 1639 (1942).
[6] K. H. MEYER (unpublished results).
[7] T. J. SCHOCH, Adv. Carbohydrate Chem. *1*, 247 (1945).

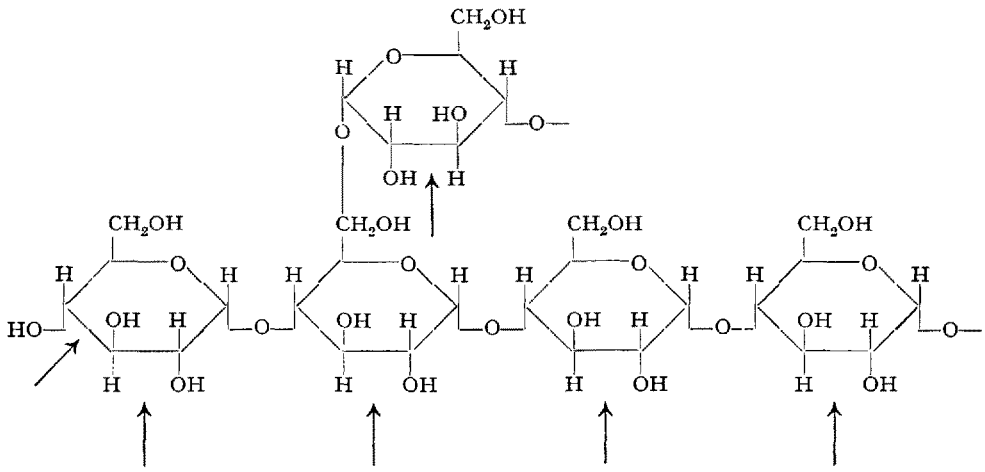

Fig. 5. – ↑ bond attacked by periodate.

plex with iodine more easily than amylopectin. One can therefore determine the amylose content by titrating with KI_3 and measuring potentiometrically the activity of the free I_2[1], alternatively, since the amylose complex is highly coloured, the light absorption of the solution may be measured[2]. The D.P. of the various fractions can be determined colorimetrically[3] by allowing dinitrosalicylic acid to be reduced by the free aldehyde groups of which there is one per molecule.

The branching factor, i.e. the number of non-reducing end-residues divided by the total number of residues, may be determined by the methylation method described above or by periodate oxidation which releases one molecule of formic acid per end residue[4]. The proportion of residues in the outer branches is determined by degradation with pure β-amylase[5]. In Table I is presented some recent results.

[1] F. L. BATES, D. FRENCH, and R. E. RUNDLE, J. Amer. Chem. Soc. *65*, 142 (1943).

[2] R. M. MCCREADY and W. Z. HASSID, J. Amer. Chem. Soc. *65*, 115 (1943).

[3] K. H. MEYER, G. NOETLING, and P. BERNFELD, Helv. chim. Acta *31*, 103 (1948).

[4] T. G. HALSALL, E. L. HIRST, and J. K. N. JONES, J. Chem. Soc. *1947*, 1399, 1427. – K. H. MEYER and P. RATHGEB, Helv. chim. Acta *32*, 1102 (1949).

[5] K. H. MEYER, P. BERNFELD, P. RATHGEB, and P. GÜRTLER, Helv. chim. Acta *31*, 1536 (1948).

The average D.P. exhibits marked differences for the same variety of starch and the starch structure varies in the leaves, fruits, and roots of the same plant[1]. The proportions of amylose and amylopectin and the D.P., determine, amongst other properties, the physical character of the starch. Cereal starches, whose concentrated pastes set rapidly to form strong translucent gels, have a very high molecular-weight A_2 fraction that is barely soluble in water in the pure state and crystallizes from hot pastes. The root, tuber, and waxy-maize starches, which set slowly to transparent gels, do not contain this fraction and therefore do not form the crystals that are responsible for the cloudiness.

The D.P. of the amylopectin controls the water absorption of the swollen grains. Potato, tapioca, and waxy maize contain amylopectin of D.P. ~1500[3] and their granules can swell 100% by weight without bursting. Maize and wheat grains which burst after absorbing 40% water contain amylopectin of D.P. ~300[3]. Further clarification of this point is given in section 7.

[1] K. H. MEYER and P. HEINRICH, Helv. chim. Acta *25*, 1038 (1942).

[2] K. H. MEYER and G. C. GIBBONS, Helv. chim. Acta *33*, 210 (1950).

[3] K. H. MEYER, G. NOELTING, and P. BERNFELD, Exper. *3*, 370 (1947).

Table I

Origin	Amylose					Amylopectin		
	Sub-fraction	%	DP.	MW.	% I_2 absorbed	DP.	MW.	% ramification
Potato . . .	A1	10	200	32,000				
	A2	12	700	110,000				
	A1 + A2	22	300	50,000	21·0	1100	178,000	5·0
Maize. . .	A1	10	250	40,000				
	A2	11	2100	340,000				
	A1 + A2	21	450	72,000	21·0	280	45,000	3·0–4·0
Tapioca . .	—	17	300	50,000	20·2	1000	162,000	4·3
Rice . . .	—	17	800	130,000	21·0	1000	162,000	3·0–4·0
Waxy maize	—	0	—	—	—	1850	300,000	4·8

Crude amylose is very easily split into fractions by prolonged treatment with water at different temperatures[1]. Only the fractions of lowest molecular-weight are soluble in cold water; intermediate fractions are soluble in hot water and separate rapidly on cooling; the highest fractions are soluble only in dilute alkali and precipitate on neutralization. Unfractionated amylose is much more soluble than the purified fractions. Its aqueous solutions are supersaturated; the amylose at once begins to form associations of a few molecules which progressively increase in size to form visible particles and finally separate as a cryptocrystalline powder known as "retrograded starch". This "aging" of fresh amylose solutions may be followed by nephelometry or by observation of the resistance to attack by α- or β-amylase[2]. A slightly turbid solution that is only slowly degraded by amylase can be made transparent and accessible to rapid amylolytic breakdown by making alkaline and subsequently neutralizing. It is convenient to designate this operation as "rejuvenation"[3].

Dilute aqueous solutions of amylose to which butanol has been added, precipitate crystalline platelets of an addition product with butanol[4]. The crystals are not, however, composed of solely one sort of molecule; they contain amyloses of different molecular weight and are in this respect analogous to the crystals obtained from degraded rubber or degraded cellulose acetate.

In contrast, amylose crystallizes from concentrated solutions e.g. in formamide, as a translucent elastic gel, very much like a gelatin gel. This phenomenon, common in the chemistry of high polymers, is due to the fact that flexible long chain molecules behave in many respects as if they were composed of "segments" which undergo thermal motions independently of other segments of the same chain. This hypothesis is one of the most important achievements of polymer chemistry. It accounts for the abnormal behaviour of solutions of high polymers[5] and for the low molecular-weights obtained by the usual methods e.g., osmotic pressure and depression of freezing point. It accounts also for the elastic force of stretched elastomers such as rubber; the tendency to return to the unstretched state is due to thermal motion of the segments[6]. It explains also the formation of a gel by crystallization[7]. Segments of different chains can arrange themselves into a lattice with evolution of heat of crystallization, while other segments are still surrounded by the solvent. Therefore different segments of one chain may finally be present in different crystallites; very small crystalline regions or micellae are thus united by molecular filaments forming the coherent elastic structure of a gel. This form of crystal is called a "fringe micelle"[1].

Amylopectin crystallizes in the same lattice as amylose, this is proved by the X-ray diagram of starches not containing amylose, e.g. waxy maize or glutinous rice; it is identical with the diagram of ordinary corn or rice starch[2]. But the whole amylopectin molecule does not fit into a lattice; the branching points remain outside the crystalline regions and only the regular parts of the chains, especially the long outer branches, participate in crystal formation. Hence branches of one amylopectin molecule will be present in different crystallites. Gel formation and, in dilute solutions, formation of clusters of fringe micellae are the forms of crystallization of amylopectin.

(6) *The Structure of Glycogen.*—The structure of glycogen was elucidated concurrently with that of amylopectin. By glycogen was originally understood a polysaccharide, hydrolyzable to glucose, which occurs in animal tissues such as liver and muscle and serves as reserve carbohydrate. Later, polysaccharides occuring in fungi, e.g. yeasts, and higher plants, e.g. maize[3], were found to be indistinguishable from glycogen, and were also so termed.

Glycogen gives a red-brown colour with iodine; it does not form granules but is dispersed in cell protoplasm. Its structure is that of a very highly branched amylopectin[4] of end group content 10%[5]. Physical measurements confirm that the molecule is very compact in shape[6]. Amylases degrade glycogen in the same way as amylopectin. Degradation with β-amylase followed by α-glucosidase and again β-amylase gives similar results as with amylopectin except that the branch segments are much shorter[7]. The irregular construction of the molecule completely prevents crystallization. In the cell, as in aqueous solution, the individual molecules are associated by mechanical entanglement to form large particles. These may be so large that they can be centrifuged from a cloudy solution. Bound protein is not, as has been thought[8], present; neither is phosphorus[9]. Synthesis and break-

[1] K. H. MEYER, P. BERNFELD, and E. WOLFF, Helv. chim. Acta *23*, 854 (1940).
[2] R. H. HOPKINS, E. G. STOPHER, and D. E. DOLBY, J. Inst. Brewing *46*, 426 (1940).
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[4] R. G. KERR and G. M. SEVERSON, J. Amer. Chem. Soc. *65*, 193 (1943).
[5] W. HALLER, Koll.-Z. *56*, 257 (1931). – K. H. MEYER, Z. physik. Chem. [B] *44*, 383 (1939).
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[3] D. L. MORRIS and C. T. MORRIS, J. Biol. Chem. *130*, 535 (1939); *154*, 503 (1944).
[4] K. H. MEYER and M. FULD, Helv. chim. Acta *24*, 375 (1941). – K. H. MEYER, Adv. Enzym. *3*, 109 (1943).
[5] W. N. HAWORTH, E. L. HIRST, and F. A. ISHERWOOD, J. Chem. Soc. *1937*, 577.
[6] H. STAUDINGER and E. HUSEMANN, Ann. Chem. *530*, 1 (1932).
[7] K. H. MEYER and M. FULD, Helv. chim. Acta *24*, 375 (1941).
[8] R. WILLSTÄTTER and M. ROHDEWALD, Z. physiol. Chem. *225*, 103 (1934).
[9] K. H. MEYER and R. JEANLOZ, Helv. chim. Acta *26*, 1784 (1943).

down of glycogen in the reserve organs is by phosphorolysis, in the intestine by amylases.

(7) *The Structure of the Starch Granule.*—Polysaccharide is not extracted from the intact granule by cold water, though those that are damaged are attacked. Hence the soluble constituents are protected by insoluble layers. An understanding of granule construction is clearly necessary for a complete comprehension of the chemical reactions of starch. Nowhere is the granule structure more important than in its behaviour towards warm water. The granules absorb water and become huge bubbles; these shrink if salt or sugar is added to the water and therefore behave like small osmotic cells[1].

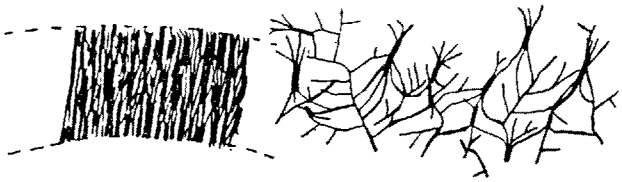

Fig. 6.–Schematic illustration of the submicroscopic structure of the starch granule. Radial section of a layer of the granule: (left) non-swollen granule; (right) swollen granule. Fine lines signify chains or parts of individual chains; thick lines signify crystalline regions.

How can one relate these phenomena to the structure? NAEGELI, MEYER, and KATZ have shown that the granule contains radially ordered needle-like crystals. This, however, only explains the optical and not the swelling and elastic properties of the granule which may be slightly elastically deformed while a true spherulite disintegrates under pressure. The crystallites must therefore be held together by some elastic element. This is accounted for by the tendency of chain polymers to form fringe micelles and, more especially, by the properties of amylopectin which can crystallize only in fringe micelles. In the intact granule the branches of different amylopectin molecules are gathered together in crystalline bundles, these bundles being themselves linked with one another by parts of amylopectin molecules which are not included in the crystal structure (Fig. 6 *b*). This fact explains why, on acid treatment, the granule becomes brittle and easily soluble: the cementing chains are attacked first so that the micelles are easily separable from each other[2].

Microscopical investigation shows that the granule is built up of concentric shells. These shells may be seen by swelling in warm water granules that have been previously heated[3]. Each shell is composed of a resistant outer layer and an inner water-soluble portion. The former does not lose its coherence when highly swollen but forms a large bubble; it consists of 90% amylopectin B_2 plus 10% amylose A_2 which form mixed crystals and are separable only on complete solution[1]. The innerportion of the shell consists of amylose A_1 which is soluble in cold water and may therefore partially dissolve from damaged granules. In waxy starches the resistant layer is entirely of amylopectin B_2 and the inner layer of soluble amylopectin B_1. A special outer membrane does not exist in any kind of starch.

According to FREY-WYSSLING[2] the refractive index of each shell decreases from inside to outside. The layers composed of amylose A_1 are thus optically dense, which is explained by their high crystallinity. On the other hand the outer layers, largely composed of amylopectin, crystallize less well because the branch points must be accomodated between crystallites; however, they are in consequence more resistant.

(8) *Swelling and Setting Phenomena.* – How is the formation on swelling of the large semipermeable membranes to be explained? They must be formed of a molecular network whose mesh allows easier passage of water molecules than of amylose A_1. Hence an osmotic pressure is set up forcing water inwards[3]; subsequently amylose slowly diffuses out and the bubbles collapse. The concentric shells are best observed in previously heated granules[4]. For a long time we speculated on the nature of the cross-links forming the net structure, and we now believe that the points of linkage are the same as in the intact granule, i.e. the crystallites. It is well known that the solubility of a small crystal depends upon its size, thus when the smaller have dissolved some larger ones still remain to form the points of linkage; at higher temperatures these too dissolve and the granule disintegrates. Clearly, the larger the amylopectin molecules that form the shell, the greater will be the extensibility of the shell before it bursts.

The swelling temperature can be raised by an annealing pre-treatment; the proportion of crystalline material increases and the individual crystallites also become larger and less soluble[5].

If starch swells in a small amount of water the shells come into contact and adhere. This also occurs if starch is heated in water without vigorous stirring. Presumably one end of a chain molecule may be fixed in a shell crystallite while the other is "dissolved" and can entangle with similar chains of adjacent shells as a result of thermal motion. Adhesive properties thus appear on a macroscopic scale. Concentrated pastes solidify on cooling and give a coherent elastic gel which is turbid if it contains amylose of high molecular-weight but is otherwise transparent. The solidification

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[2] A. FREY-WYSSLING, Protoplasma *25*, 261 (1936).
[3] K. H. MEYER and M. FULD, Helv. chim. Acta *25*, 391 (1942.)
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Table II

	β-amylase from		α-amylase from					
	Sweet potatoes[1]	Malt[2]	Malt[3]	Asperg. Oryzae[4]	Bacillus subtilis[5]	Swine pancreas[6]	Human pancreas[7]	Human saliva[8]
Activity per mg. N.	2,500[12]	1,665	2,350	2,400	3,600	4,000	6,200	6,200
Activity per mg. enzyme	378	235	315	310	500	630	980	980
% N	15·1	14·1	13·4	12·9	14	15·8	15·8	15·8
% P			0	0	0	0·05	0	0
SH-groups	+	+	–[14]	–	–	–	–	–
Optimum pH	4–5	5·2	4·7–5·4[14]	5·5–5·9	5·3–6·8	6·9	6·9	6·9
Optimum stability pH		4·0–8·0	4·9–9·1[14]	5·5–8·5	4·8–8·5	7–8·5	4·8–11	4·8–11
% solubility at 2° pH 7		15	15[14]	10	6	0·6	0·3	0·3
Activation energy[9]		16,200 (0–20°) 5,530 (20–50°)	7,050[14]	10,650		13,500	13,500	13,500
Molecular weight	152,000[13]		59,500			45,000[15]		
Electrophoretic mobility (pH 7·9, $\mu = 0{\cdot}1$) $\mu \cdot 10^{-5}$ cm^2 · volt^{-1} · s^{-1}		2·4	3·1[14]		3·1	3·1	3·2	3·2
Isoelectric point	4·77[13]	6·0	5·7[14]	4·2		5·2–5·6	5·2–5·6	5·2–5·6
Turnover number[10]			17,000			25,000		
Absorption spectrum (mμ)		280	280	280	280	280	280	280
Activation by Cl^-	–	–	–	–	±	+	+	+
Activation by Ca^{++}	–	–	+	–	–	–	–	–
Ratio *saccharogene* activity (±0·2)[11] dextrinogen	61	62	9·8	9·8	9·8	9·8	9·6	9·6

is hampered mechanically by the presence of very large amylopectin molecules. Limited acid hydrolysis of starch may therefore improve the gel-forming properties of e.g. potato starch.

The influence of the phenomenon of crystallization on the general properties of starch can hardly be overemphasized. In fresh native starch crystallization is incomplete; it can increase over long periods the swelling temperature being thereby raised. The effect of crystallization in bread is very conspicuous, for staling is nothing other than a crystallizing of fringe-micelles[16].

(9) *Pure Amylases.*—Before treating the enzymic degradation of starch it seems convenient to summarize the progress made in the purification of the enzymes themselves.

The choice of method to be adopted for enrichment and purification is rather empirical; fractional precipitation at 0° with acids, acetone, ammonium sulphate, or other salts, and denaturation by heat or agitation and other devices are applied to remove unwanted proteins. At each stage the enrichment is controlled by analysis of nitrogen content and measurement of enzyme activity. Homogeneity is tested for by electrophoresis and when it is attained patient attempts are made to induce crystallization; finally the enzyme is purified by repeated recrystallization.

β-Amylases liberate maltose in the downward rotating β-form; they attack the starch polysaccharide from the non-reducing end of the molecule with the immediate formation of maltose. β-Amylases have been found only in plants; in malt, sweet potato and soya bean. The first crystalline amylase to be isolated (β-amylase) was obtained by BALLS[1] in 1946 from the sweet potato (*Ipomoea batatas*) (Fig. 7); the purification and crystallization of the much longer known β-amylase of malt has only recently been achieved at Geneva[2].

α-Amylases, so called because the aldehydic groups thereby liberated are in the upward rotating α-form,

[1] A. K. BALLS, R. L. THOMPSON, and M. K. WALDEN, J. Biol. Chem. *163*, 571 (1946); *173*, 9 (1948).

[2] ED. H. FISCHER, K. H. MEYER, G. NOELTING, and A. PIGUET, Arch. Biochem. *27*, 235 (1950); A. PIGUET and ED. H. FISCHER, Helv. chim. Acta *35*, 257 (1952).

[3] S. SCHWIMMER and A. K. BALLS, J. Biol. Chem. *179*, 1063 (1949).

[4] ED. H. FISCHER and R. DE MONTMOLLIN, Helv. chim. Acta *34*, 1987, 1994 (1951).

[5] KURT H. MEYER, M. FULD, and P. BERNFELD, Exper. *3*, 411 (1947), and unpublished results.

[6] KURT H. MEYER, ED. H. FISCHER, and P. BERNFELD, Helv. chim. Acta *30*, 64 (1947). – ED. H. FISCHER and P. BERNFELD, Helv. chim. Acta *31*, 1831 (1948).

[7] ED. H. FISCHER, F. DUCKERT, and P. BERNFELD, Helv. chim. Acta *33*, 1060 (1950). – P. BERNFELD, F. DUCKERT, and ED. H. FISCHER, Helv. chim. Acta *33*, 1064 (1950).

[8] K. H. MEYER, ED. H. FISCHER, A. STAUB, and P. BERNFELD, Helv. chim. Acta *31*, 2158, 2165 (1948).

[9] ED. H. FISCHER, Helv. chim. Acta (to be published).

[10] Number of glucosidic bond splitted per minute and per 1 mole of enzyme.

[11] P. BERNFELD and M. FULD, Helv. chim. Acta *31*, 1423 (1948).

[12] According to our own experiments.

[13] S. ENGLARD and TH. P. SINGER, J. Biol. Chem. *187*, 213 (1950).

[14] ED. H. FISCHER and C. H. HASELBACH, Helv. chim. Acta *34*, 325 (1951).

[15] C. E. DANIELSSON, Nature *160*, 899 (1947).

[16] F. R. KATZ, Z. physiol. Chem. [A] *150*, 37 (1930); *169*, 321 (1934).

[1] A. R. BALLS, R. L. THOMPSON, and M. K. WALDEN, J. Biol. Chem. *163*, 571 (1946); *173*, 9 (1948).

[2] K. H. MEYER, ED. H. FISCHER, and A. PIGUET, Helv. chim. Acta *34*, 316 (1951). – A. PIGUET and ED. H. FISCHER, Helv. chim. Acta *35*, 257 (1952).

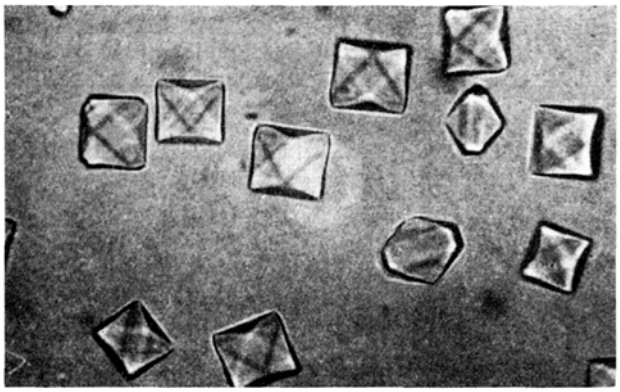


Fig. 7.

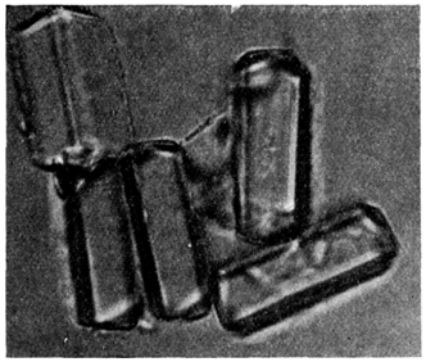


Fig. 8.

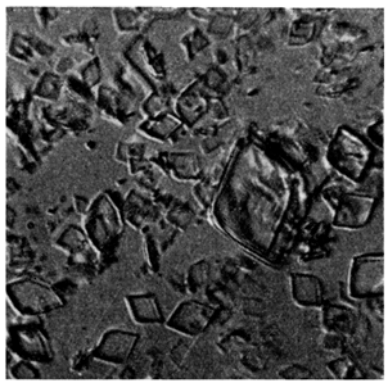


Fig. 9.

Fig. 10.

initially degrade the starch polysaccharide into large fission products. The first to be purified[1], in the form of beautiful small crystals, was swine pancreas amylase[2]; later, crystalline forms of "ptyalin" (the α-amylase of human saliva[3]) (Fig. 8) and of human pancreas amylase were obtained[4] (Fig. 9). These last two cannot be distinguished from each other but swine pancreas amylase (Fig. 10) is quite certainly different, so that we assume there is only one human amylase. Thus it would appear that it is the species and not the particular organ which determines the constitution of the enzyme.

Of the vegetable α-amylases, malt α-amylase[5] (Fig. 11) and the α-amylases from *Bacillus subtilis*[6] (Fig. 12) and *Aspergillus oryzae*[7] (Fig. 13) have been obtained crystalline. The properties of the amylases are compared in Table II.

(10) *Action of pure amylases on pure amylose and amylopectin.*—The purification of the two constituents of starch–linear amylose and branched amylopectin,– and of the amylolytic enzymes, made it possible to investigate the effects of these substances on each other. This is necessary for a clearer picture of the degradation of starch because in mixed amylases, e.g. malt, many reactions are super-imposed.

Our investigations confirmed the earlier work on β-amylase degradation[8], the enzyme acts from the non-reducing end groups and completely degrades amylose. The findings of some authors[9] that amylose is not fully degraded are probably due to the use of impure samples. Amylopectin is attacked as illustrated by Figure 4; the branch points and, in the case of tuber-starches, the phosphate groups arrest the action of β-amylase. It can be shown that the "limit dextrin" contains the same number of end groups and branch

[1] In a preliminary note of 1931, M. L. CALDWELL, L. E. BOCHER, and H. C. SHERMAN (Science *74*, 37 [1931]) reported the obtention of a crystalline material, very "unstable", of an "enzymic activity almost as high as the maximum observed" by them and claimed to be swine pancreatic α-amylase (?). This material can easely be obtained in the first or second stages of the purification of the enzyme and is certainely not crystalline amylase. It simply consists of inactive proteins, ev. scission products of amylase (ED. H. FISCHER and P. BERNFELD, Helv. chim. Acta *31*, 1839 [1948]), disclosing a certain activity through adsorption of the enzyme, a fact which is easy to show by mere recrystallizations. Crystalline amylase is stable and can be recrystallized.

[2] K. H. MEYER, ED. H. FISCHER, and P. BERNFELD, Exper. *2*, 362 (1946); *3*, 106 (1947). – ED. H. FISCHER and P. BERNFELD, Helv. chim. Acta *31*, 1831 (1948).

[3] K. H. MEYER, ED. H. FISCHER, A. STAUB, and P. BERNFELD, Helv. chim. Acta *31*, 2158 (1948).

[4] K. H. MEYER, ED. H. FISCHER, P. BERNFELD, and F. DUCKERT, Arch. Biochem. *18*, 203 (1948). – ED. H. FISCHER, F. DUCKERT, and P. BERNFELD, Helv. chim. Acta *33*, 1060 (1950).

[5] S. SCHWIMMER and A. K. BALLS, J. Biol. Chem. *176*, 465 (1948).

[6] K. H. MEYER, M. FULD, and P. BERNFELD, Experientia *3*, 411 (1947).

[7] ED. H. FISCHER and R. DE MONTMOLLIN, Helv. chim. Acta *34*, 1987, 1994 (1951). – L. A. UNDERKOFLER and D. K. ROY, Cereal Chem. *28*, 18 (1951).

[8] K. H. MEYER, ED. H. FISCHER, and P. F. SPAHR, Helv. chim. Acta (in press).

[9] S. PEAT, S. G. PIRT, and W. J. WHELAN, J. Chem. Soc. *1952*, 705.

points as the original amylopectin; the enzyme has only been able to attack the long branches[1]. A schematic representation of "limit dextrin" is given.

The investigation of the α-amylase degradation is more difficult. Attack is along the chain and the first products are fragments of high molecular weight, then dextrins of lower molecular weight appear including maltotetraose, maltotriose and branched tri- and tetrasaccharides, the final products are maltose, isomaltose, and glucose.

To estimate these mixtures, the specific fermentation of sugars can be used[2]. The amylase is killed by heating, then *Torula monosa* is added, which ferments glucose only. By estimating the reducing before and after this fermentation the glucose content is obtained by difference. Then *Saccharomyces chodati* is added, whereby glucose, maltose, and much more slowly, isomaltose are fermented. Small amounts of sugars may be detected by paper chromatography. The course of degradation is followed by reductometric determinations and a complete analysis of the products is carried out from time to time.

Investigations on the action of pure pancreas amylase and pure malt α-amylase respectively on pure amylose gave the following results[3].

At very high enzyme concentrations the reaction proceeds approximately at the rate of a monomolecular reaction until 60% maltose and 40% maltotriose are formed; the latter is then split into glucose and maltose by a very slow reaction. The end products are 13% glucose and 87% maltose. At medium enzyme concentrations the reaction decelerates at an earlier stage; this slowing down is due to three different influences.

(a) *Ageing of the amylose*, i.e. formation of submicroscopic associations and crystallites which are resistant to enzymic attack[4]. After rejuvenation and subsequent addition of enzyme the reaction starts again[5].

(b) *The affinity of the enzyme* for the polysaccharide diminishes with decreasing D. P. of the latter[6]. It is generally believed today that the rate of enzymic fission is determined by two factors, the concentration of the enzyme-substrate (in this case amylase-polysaccharide) compound and its decomposition rate. Now according to the law of mass action, ES, the concentration of the compound, depends on the equilibrium

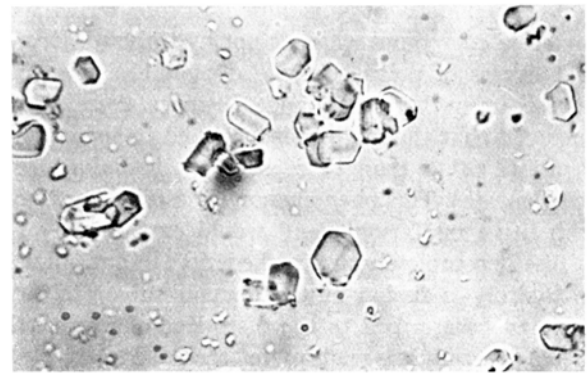


Fig. 11.

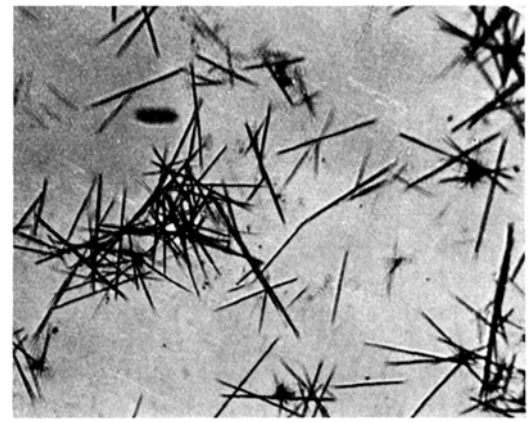


Fig. 12.

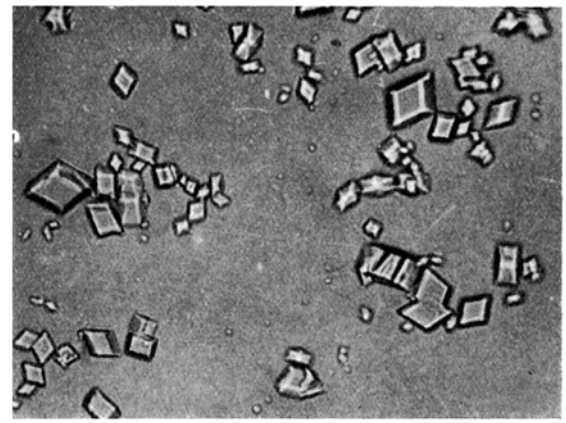


Fig. 13.

constant K and the concentrations of both reactants, enzyme E and substrate S:

$$\frac{ES}{(E-ES)\,(S-ES)} = K.$$

Now if K falls with decreasing degree of polymerization of the substrate present, then the rate of fission must continually decrease as the D. P. is continually diminished[1].

[1] ED. H. FISCHER (in press).

[1] K. H. MEYER, M. WERTHEIM, and P. BERNFELD, Helv. chim. Acta *24*, 212 (1941).

[2] A. J. KLUYVER, Biochem. suikerbepalingen, Leiden (1914). – K. H. MEYER and W. F. GONON, Helv. chim. Acta *34*, 290 (1951).

[3] K. H. MEYER and W. F. GONON, Helv. chim. Acta *34*, 294 (1951).

[4] O. E. STAMBERG and C. H. BAILEY, Cereal Chem. *16*, 330 (1939). – R. M. HOPKINS, E. G. STOPHER, and D. E. DOLBY, J. Inst. Brewing *46*, 426 (1940).

[5] P. BERNFELD and H. STUDER-PECHA, Helv. chim. Acta *30*, 1895 (1947).

[6] K. MYRBÄCK and N. O. JOHANSEN, Arkiv Kemi Min. Geol. (A) *20*, (6) (1945). – K. MYRBÄCK and E. SILHBOM, Arkiv. Kemi *1*, [1] (1949). – R. M. HOPKINS, Adv. in Enzymology *6*, 389 (1946).

(c) *The inhibition by maltose.* Maltose forms with amylase a compound which is not hydrolyzed. Even a great excess of polysaccharide does not displace the maltose from this compound. It must therefore be concluded that the maltose is attached to the enzyme by groups other than those involved in linkage with the substrate[1]. This phenomenon is described as "non-competitive inhibition", and would explain why the maltose is completely unattacked, for, in our opinion, the hydrolytic fission of the enzyme-substrate complex may be ascribed to the distortion of a glucosidic linkage, whereby it is made more accessible to attack by water. If, however, maltose is differently linked to the enzyme, the pre-condition for hydrolytic fission is lacking.

The reaction of pure α-amylase with pure amylopectin proceeds similarly to that with amylose. In a relatively rapid reaction, unbranched and branched oligosaccharides are formed. These slowly undergo fission into maltose, glucose and isomaltose. End products are 72% maltose, 19% glucose and 9% isomaltose. All branch points (α-1:6′ bonds) reappear finally in the form of isomaltose[2].

The affinity of the enzyme for the branched polysaccharides is very small; the considerable diminution of affinity for the substrate during progressive hydrolysis leads to a very marked deceleration of the reaction.

(11) *The degradation of starch.*—(a) *By Malt.*—In this process the four reactions discussed above i.e. the breakdown of amylose by α- and β-amylase and the degradation of amylopectin by the same enzymes proceed simultaneously and influence each other. The following considerations apply. β-Amylase degrades dissolved amylose completely and amylopectin to 60–70% of fermentable sugars, 30% of a nonfermentable limit dextrin being formed. α-Amylase degrades amylose initially only to 70% fermentable sugars and 30% non-fermentable maltotriose. Amylopectin is degraded only to about 60% sugars in the initial reaction. These facts show that the yield in sugars is improved when a mixture of both amylases is used. In β-amylase degradation the limit β-dextrin is split up by addition of α-amylase and the reducing end groups thus liberated can undergo further β-amylase attack. In the case of α-amylase action the addition of β-amylase increases the percentage of maltose and decreases that of triose formed.

Here too, maltose functions as an inhibitor. This explains why the degradation proceeds more rapidly in the presence of yeast; maltose is fermented and the inhibition ceases.

If a high yield of fermentable sugars is desired it is advantageous to employ a large quantity of β-amylase admixed with a small quantity of α-amylase. In contrast, an α-amylase predominance results in a higher yield of non-fermentable oligosaccharides. This condition is achieved in the brewing industry where the β-amylase in green malt is destroyed to a large extent by the torrefaction process.

In the usual starch pastes, granular residues exist along with dissolved components: α-amylase "liquefies" because it will split up the polysaccharides at any point of the surface of a cluster and even of the intact granule. In contrast β-amylase action is confined to the end groups which may be hidden inside. This seems to be the case in the intact starch granule since it is not attacked by pure β-amylase. In a paste the course of reaction will therefore depend on the degree of disintegration.

In technical processes other factors such as temperature, pH, ion content, and the presence or absence of yeast also influence the course of degradation. The actual process can not therefore be forecast and must be established experimentally for each individual case.

(b) *In the human body.*—The attack on starch begins in the mouth and is completed in the intestine by pancreatic juice. Saliva and pancreas contain only α-amylase as amylolytic enzyme; the belief that maltase is present in saliva is incorrect, – the glucose found is produced from maltotriose. A maltase is however secreted by the mucous membrane of the intestine to convert maltose to glucose. How the branched oligo-saccharides are degraded in the intestine is not yet known.

(12) *Other Amylases.*—As already stated the α-1:6′ links are not attacked by the common amylases. However, lately some α-1:6′ amylases have been described —they would be better known as "isoamylases". Isoamylases are found in autolysed brewers yeast[1], in broad bean, potato[2] and other plants[1].

Also of interest is a "glucoamylase" which was obtained from *aspergillus niger* and *rhizopus delemar*; it is analogous to β-amylase and removes one glucose unit at a time from the non-reducing end-residues to the branch points[3]. This enzyme cannot attack glucose units linked by 1:6′ bonds, but there are enzymes which perform this function. Such "amyloglucosidases" occur in autolysed yeast[4] and in muscle[5].

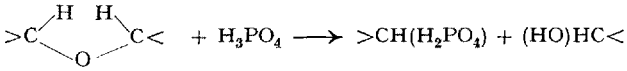
(13) *Phosphorolytic Degradation and Synthesis of Starch Polysaccharides.*–The equilibrium of the reaction:

Polysaccharide + Water → Breakdown products,

lies practically completely on the side of the products. It is therefore impossible to synthesise polysaccharide from glucose or maltose by means of amylases. A great advance was made when PARNAS[6] found that glycogen, which is very similar to amylopectin, may be scinded

[1] S. SCHWIMMER, J. Biol. Chem. *186*, 181 (1950).
[2] K. H. MEYER and W. F. GONON, Helv. chim. Acta *34*, 294 (1951).
[1] B. MARUO and T. KOBAYASHI, Nature *167*, 606 (1951).
[2] P. N. HOBSON, W. J. WHELAN, and S. PEAT, Biochem. J. *47*, 39 (1950).
[3] R. W. KERR, F. C. CLEVELAND, and W. J. KATZBECK, J. Amer. Chem. Soc. *73*, 3916 (1951). – L. L. PHILLIPS and M. L. CALDWELL, J. Amer. Chem. Soc. *73*, 3563 (1951).
[4] K. H. MEYER and P. BERNFELD, Helv. chim. Acta *25*, 404 (1942).
[5] G. T. CORI and J. LARNER, J. Biol. Chem. *188*, 17 (1951).
[6] J. K. PARNAS and T. BARANOWSKI, C.r. Soc. Biol. *121*, 282 (1936).

by phosphorolysis. In presence of phosphorylase, the glucosidic linkages are split with the addition of phosphoric acid, not water



CORI[1] found that glucose: 1:phosphate, since known as Cori-ester, is produced. SCHÄFFNER and SPECHT[2], KIESSLING[3], and CORI[4] showed that the reaction is reversible; from Cori-ester a glycogen-like polysaccharide may be obtained.

The application of this discovery to the plant world is due to HANES[5] who showed that phosphorylase is widely distributed and assists in producing a polysaccharide from glucose phosphate. This polysaccharide was later found to be amylose. The plant phosphorylases can only scind or re-form 1:4′ bonds.

From this synthetic amylose one can obtain a branched polysaccharide with the properties of amylopectin by the action of the "Q" enzyme discovered by BOURNE and PEAT[6]. How the enzyme functions is not yet quite clear. It converts by transglucosidification[7], α-dextrins of an average chain length of more than 42 glucose units to amylopectin[8].

We found a considerable concentration of phosphorylase in potato shoots and hence consider it very probable that starch is transported from the tuber to the points of growth, and conversely, from leaf to reserve organ in the form of glucose:1:phosphate. Whether glucose:1:phosphate is an intermediate in the actual synthesis of leaf starch is not known.

(14) *Amylosucrase, Amylomaltase and Macerans Amylas.*—Several enzymes may both degrade and synthesize polysaccharides of the starch group by transglucosidification, that is, migration of glucosidic bonds, no hydrolysis or phosphorolysis being involved. From cultures of *Neisseria perflava* an enzyme termed amylosucrase has been obtained which transforms sucrose into fructose and an amylopectin-like substance (HEHRE[9]).

$$\underset{\text{sucrose}}{n\ C_{12}H_{22}O_{11}} \longrightarrow (C_6H_{10}O_5)_n + \underset{\text{fructose}}{n(C_6H_{12}O_6)}$$

A similar enzyme, amylomaltase, transforms maltose into a polysaccharide of the starch type and glucose; it occurs in *Escherichia coli* (MONOD)[10].

As early as 1903 SCHARDINGER[1] obtained crystalline dextrins (so-called SCHARDINGER dextrins) by the degradation of starch with *Bacillus macerans*. The reaction is reversible and due to an enzyme[2]. The "dextrins" are closed ring compounds with 6, 7 or 8 glucose residues respectively joined to one another by α-1:4′ linkages[3]. Since these structures are not present in the original starch, care is obviously always necessary in interpreting results obtained with impure enzymes.

Concluding Remarks

We shall now review the general picture of the structure of starch and in so doing will proceed from the structure of the granule to that of the molecule.

Under the microscope one observes well formed granules which show in polarised light the black cross characteristic of spherulites. The granules are built up of concentric shells each of which consists of an outer water-resistant layer that transforms gradually into an inner layer partially soluble in cold water. Both layers contain radially arranged needle-like crystallites. The resistant outer layer consists of 90% of a very highly branched polysaccharide, amylopectin B_2, and 10% of a high molecular-weight linear polysaccharide, amylose A_2. They exist as mixed crystals of submicroscopic dimensions. The numerous branch points of the amylopectin molecules are not in the crystals proper but in the amorphous regions which form an elastic cement between the crystallites. Just as the crystals are connected by molecular fibres so the individual molecules are linked in a lattice. The less resistant inner layers consist of well crystallized low molecular-weight amylose A_1. The outer layers form very fine meshed networks that expand when some of the crystallites dissolve in warm water. Water then enters and the inner layers are attacked, dissolved amylose diffusing out. However, only the small easily soluble crystallites are destroyed in warm water, the larger ones remain to preserve the identity of the swollen bubble-shaped networks. Thus the network is the more extensible the larger its constituent amylopectin molecules. When swollen starch granules come into contact the free ends of molecules from adjacent granules intertwine, and the granules agglutinate. Concentrated pastes of cereal starch set rapidly to very cloudy gels, due to the presence of a very high molecular-weight amylose (A_2) which easily formes large crystallites. This amylose is not present in potato and waxy maize starches whose pastes set slowly to transparent gels containing much smaller crystals.

Starch granules that are very rich in amylose (e.g. wrinkled pea), dissociate on heating in water without

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[8] S. NUSSENBAUM and W. Z. HASSID, J. Biol. Chem. *196*, 785 (1952).
[9] E. J. HEHRE, J. Biol. Chem. *177*, 267 (1949).
[10] J. MONOD and A. M. TORRIANI, C. r. Acad. Sci. *227*, 240 (1948).

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[2] E. B. TILDEN and C. S. HUDSON, J. Amer. Chem. Soc. *61*, 2900 (1939).
[3] D. FRENCH and R. E. RUNDLE, J. Amer. Chem. Soc. *64*, 1651 (1942).

the typical swelling phenomena. The glutinous starches, which give a purple colour with iodine, contain a low molecular-weight amylopectin in place of the low molecular-weight amylose. Degradation by α- and β-amylases yields results in good agreement with this picture of the constitution and structure of the starch polysaccharides. Intact granules and even granule fragments are not degraded by β-amylases which attack only from the ends of the molecules. α-Amylases, which attack at any part of the molecule, can slowly hydrolyse the intact granule. Neither amylase is able to break the isomaltose link. The type of residue found in a digest depends upon the degree of degradation, proportion of each enzyme, temperature, and content of Ca and Cl' ions.

We believe that this picture of the structure and constitution of starch covers all aspects of starch chemistry. This end is only to be arrived at by utilising the methods, theories, and data of all branches of natural science—from botany, from organic, physical, and polymer chemistry, from physics, and from enzymology. The frontiers between the individual branches of science are ever tending to disappear; there will remain a unified Science of Nature.

Résumé

Le présent travail retrace l'évolution qu'a subie la chimie de l'amidon de son origine à nos jours.

Avant de faire le point de nos connaissances actuelles sur ce sujet, nous avons jugé indispensable de rappeler la part prise par les botanistes, les colloïdologistes, les chimistes organiciens et les enzymologues à la solution de ce vaste problème. Car ce n'est qu'en faisant la somme des travaux poursuivis dans tant de domaines différents que l'on arrive à une image claire couvrant tous les aspects de la structure et de la constitution de l'amidon.

Les grains d'amidon sont constitués de couches concentriques dont les faces externes sont moins solubles dans l'eau que les faces internes. Ces couches contiennent des cristallites arrangés radialement et responsables du phénomène de la croix noire. Les couches résistantes sont constituées de 90% d'un polysaccharide fortement ramifié, l'amylopectine B_2, et de 10% d'un polysaccharide linéaire de poids moléculaire élevé, l'amylose A_2; ils forment ensemble des cristaux mixtes, quoiqu'une partie de la molécule d'amylopectine subsiste à l'état amorphe, donnant une certaine élasticité au système. Les couches internes, facilement solubles, sont constituées d'amylose A_1 de poids moléculaire bas, bien cristallisée. Dans l'eau chaude, les cristallites les plus solubles se dissolvent et l'amylose A_1 diffuse à l'extérieur pendant que les couches les plus résistantes se gonflent; elles seront d'autant plus extensibles que l'amylopectine qu'elles contiennent sera de poids moléculaire plus élevé. Les propriétés des différents amidons et celles de leurs empois dépendent essentiellement de la nature des polysaccharides présents: elles ont été longuement exposées.

En fin de cet article, nous décrivons les propriétés des enzymes amylolytiques, leur mode d'action et le rôle qu'elles peuvent avoir sur la dégradation et la synthèse des polysaccharides de l'amidon.

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Über vierdimensionale Einsteinräume

Wir betrachten geschlossene vierdimensionale Mannigfaltigkeiten M^4, auf denen eine komplex-analytische Struktur samt einer hermiteschen Metrik

$$ds^2 = g_{j\bar{k}}\, dz^j\, d\bar{z}^k$$

mit

$$R_{j\bar{k}} = -c\, g_{j\bar{k}} \qquad (1)$$

gegeben ist (Einsteinsche Metrik). Die der Metrik zugeordnete alternierende Form ist

$$e = \frac{1}{2\pi\, 1} g_{j\bar{k}}\, dz^j \wedge d\bar{z}^k,\ de = 0.$$

Die Metrik sei so normiert, dass das Volumen der Mannigfaltigkeit, berechnet durch $\int_M e \wedge e$, gleich 1 wird. Im Ursprung eines geodätischen Koordinatensystems ist dann

$$R_{1\bar{1}1\bar{1}} = R_{2\bar{2}2\bar{2}} = a,$$
$$R_{1\bar{2}2\bar{1}} = R_{2\bar{1}1\bar{2}} = R_{1\bar{1}2\bar{2}} = R_{2\bar{2}1\bar{1}} = b.$$

Die Chernschen Klassen sind ganzzahlige Kohomologieklassen[1], die sich in diesem speziellen Fall durch e berechnen lassen:

$$\Psi_1(z_2) = -c\int_{z_1} e, \quad \Psi_2(z_4) = \int_{z_4} \lambda e \wedge e.$$

[1] S. S. CHERN, Ann. Math. *47*, 85 (1946).