516. *Physicochemical Studies on Starches. Part V.* The Effect of Acid on Potato Starch Granules.*

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The degradative effect of 0·2M-hydrochloric acid at 45° on the granular structure and molecular size of the components of two varieties of potato starch has been examined. The fractionated starch components were characterised by iodine-binding power, osmotic pressure, viscosity, and sedimentation. The mode of acid attack was similar for both samples. Under the conditions used, the granular structure was apparently unchanged, but both the amylose and the amylopectin component were degraded. The rates of degradation indicated that the amylopectin underwent preferential hydrolysis. The implications of these results with regard to granular structure are discussed.

Notwithstanding the large amount of work carried out on starch, little is yet known of the fine structure of the granule and the manner in which amylose and amylopectin are incorporated into it. It has been suggested that amylose is found in the granule preferentially at the centre, but it has also been maintained that it is evenly distributed throughout.[1] One approach to this problem is to study the effect of acid-treatment on the granule by estimating, after fractionation, the molecular size of the components. If the granule has an outer layer of amylopectin, the latter should be preferentially hydrolysed, whereas if there is uniform distribution both components should be degraded. In studies of the granular structure of potato starch, we have therefore examined such effects. Previous investigations along these lines have had contradictory results: Meyer and Menzi [2] reported that acid-treatment of potato and maize starch gives simultaneous degradation of both components, whilst Kerr [3] reported preferential degradation of amylopectin in maize starch. However, Meyer and Menzi [2] measured molecular weights by the dinitrosalicylic acid method, which has been shown to be unreliable for absolute molecular weights,[1] and Kerr [3] evaluated his osmotic data for amylopectin solutions by a method which has not yet been substantiated.[1] The studies reported here were undertaken in an effort to clarify the position, as all physical properties of the granule depend ultimately on granular structure and any method of tackling the latter problem is thus important.

Experimental Methods

Isolation and Purification of Potato Starch.—Potatoes (var. Redskin and Arran Banner severally) were thickly peeled, sliced, and then minced into ethanol to inhibit enzymic activity. After extraction with ethanol for 2 min. in an " Atomix " blendor, the pulp was filtered through muslin, and the filtrate *immediately* centrifuged. The supernatant liquid was discarded, and the starch washed by repeated sedimentation in 0·1M-sodium chloride. The residual pulp was then re-extracted 3—4 times with saline in the blendor. (Although extraction was obviously incomplete, the residual pulp was then discarded.) Starch rapidly sedimented from the filtrates. The starch-products were combined, suspended in 0·1M-sodium chloride, and shaken with toluene ($\frac{1}{10}$ vol.) overnight to denature protein.[4] After the granules had been allowed to sediment, the coagulated protein–toluene layer was discarded, and the extraction repeated four times to yield pure starch, which was stored in 0·1M-sodium chloride under toluene at 0° (Arran Banner) or washed free from salt and stored under methanol at 0° (Redskin) (Found, for both samples: N, 0·02%).

Characterisation of Starch Products.—The efficiency of fractionation and the overall effect of acid-treatment on the granules was followed by measuring the amount of iodine bound by

* Part IV, *J. Polymer Sci.*, 1957, in the press.

[1] For a review see Greenwood, *Adv. Carbohydrate Chem.*, 1956, **11**, 335.
[2] Meyer and Menzi, *Helv. Chim. Acta*, 1953, **36**, 702.
[3] Kerr, *Stärke*, 1952, **4**, 39.
[4] Greenwood and Robertson, *J.*, 1954, 3769.

the various starch products. In view of the insensitivity of optical-absorption methods, the binding power was measured in the semimicro differential-titration apparatus previously described.[5] Titration conditions were: [iodide], 0·01M; pH, 5·85; temp., 20°.

The total free iodine in the starch solution was plotted against the mg. of iodine bound per 100 mg. of starch, and estimations of iodine affinity (the preferential uptake of iodine by the linear amylose component) were made by extrapolating the linear portion of the titration curves to zero free-iodine concentration. Calculations of the approximate percentage of amylose were made from the observed iodine affinity on the assumption that pure potato amylose bound 19·5% of iodine under these conditions.

Treatment of Granules with Acid.—Potato starch (10 g.; var. Redskin, washed free from salt) was stirred slowly (20 revs./min.) in 0·2M-hydrochloric acid (500 ml.) at 45° under oxygen-free nitrogen. Portions (125 ml.) were removed after 1, 2, 3, and 4 hr. severally (Expt. I). The starch was immediately washed 8—12 times with distilled water in the centrifuge until free from acid and then shaken with methanol for 24 hr. The samples were stored under methanol.

The above was repeated (Expt. II), except that starch samples (var. Arran Banner) were removed at intervals of ½, 1, 2, 3, 4, and 24 hr.

Estimation of Amount of Granule Solubilised by Acid.—A 2% suspension of starch in 0·2M-hydrochloric acid was kept at 45° for 1 hr. under nitrogen. A portion of the suspension was removed, centrifuged, and reduced in volume, and the amount of glucose obtained on hydrolysis by 3N-sulphuric acid for 2 hr. at 100° was estimated by the alkaline ferricyanide–ceric sulphate method of Lampitt, Fuller, and Coton.[6] The starch was re-treated with acid for 3 hr., and the additional glucose found in the supernatant liquors estimated. The residual starch granules were weighed.

To investigate whether oligosaccharides were present inside the granule after acid-treatment, starch (2 g.; treated with 0·2M-hydrochloric acid for 4 hr.) was gelatinised at 70° in water (400 ml.) for 1 hr. under nitrogen, and amylose precipitated with butan-1-ol. The supernatant liquors were then reduced to dryness, extracted with 50% methanol, and examined chromatographically (solvent: butan-1-ol–benzene–pyridine–water: 5 : 1 : 3 : 3; top layer; development time, 72 hr. at 18°).

Fractionation of Acid-treated Starches.—A slurry of starch in methanol was carefully added to vigorously stirred boiling water (500 ml.) under nitrogen, and boiling continued for 1½ hr. (Expt. I). (In Expt. II, the boiling was continued for 1 hr.) The solution was then allowed to cool to 60°, powdered thymol (0·5 g.) added, and the mixture set aside at room temperature (18°) for 3 days before the amylose–thymol complex was removed on the Sharples super-centrifuge. The amyloses were then purified by recrystallisation twice (Expt. I) from hot butan-1-ol saturated water, and were stored as the butan-1-ol complexes. Only one recrystallisation was carried out in Expt. II. Solid amyloses were isolated by stirring the butanol complex several times with butan-1-ol and then drying it *in vacuo* at 75°.

The amylopectin-containing supernatant liquors from the thymol–precipitates were freeze-dried directly, refluxed with methanol to remove thymol (3 times; 1½ hr. each), redispersed in water, and freeze-dried. In Expt. II, the supernatant liquors were extracted twice with ether before freeze-drying. This procedure yielded more soluble products.

Fractionation of Original Starch.—A 0·5% solution of starch was dispersed as above (1½ hr. for Expt. I and 1 hr. for Expt. II). After formation of the thymol complex, the amylose was reprecipitated three times with butan-1-ol and stored as this complex. Amylopectin was obtained as above.

Acetylation of Amyloses.—A portion of the butanol complexes of each of the amyloses from Expt. I was acetylated under conditions of minimum degradation [4] with pyridine and acetic anhydride at room temperature.

Estimation of Length of Unit Chain for Amylopectins.—The amylopectins from Expt. II were oxidised with sodium metaperiodate at 2° by Potter and Hassid's method [7] with the modification [8] that the liberated formic acid was estimated by titration to pH 6·25. The yield of formic acid was constant after 25 hr.

[5] Anderson and Greenwood, *J.*, 1955, 3016.
[6] Lampitt, Fuller, and Coton, *J. Sci. Food Agric.*, 1955, **6**, 656.
[7] Potter and Hassid, *J. Amer. Chem. Soc.*, 1948, **70**, 3488.
[8] Anderson, Greenwood, and Hirst, *J.*, 1955, 225.

Measurements of Limiting Viscosity Number.—The specific viscosity ($\eta_{sp.}$) of the polysaccharide solutions was determined at several concentrations at 22·5°, and the limiting viscosity number $[\eta]$ determined graphically from the relation $[\eta] = \lim_{c \to 0}(\eta_{sp.}/c)$. Concentrations were expressed [9] as g./ml. Measurements were made on both of the unsubstituted components in M-potassium hydroxide, and on the acetylated amyloses in chloroform solution. Techniques were as previously described.[4]

Solution and concentration estimation was as follows:

(a) *For amyloses.* A portion of well-centrifuged butanol complex was dissolved directly in M-potassium hydroxide. Concentrations were then determined by hydrolysing a portion of the diluted resultant solution, and determining the reducing power as above.[6] The method was calibrated by standard solutions of glucose. Preliminary experiments indicated a recovery of 98—102% of glucose for hydrolysed "AnalaR" soluble starch. {Control experiments showed that the limiting viscosity number for a given sample was the same within experimental error whether solution had been achieved *via* the butanol complex or directly from the dried solid. However, the value of Huggins's constant (k') in the relation

$$\eta_{sp.}/c = [\eta] + k'[\eta]^2 c$$

varied.}

(b) *For amylopectins.* Solutions were prepared by weight.

(c) *For amylose acetates.* Concentrations were determined after measurements as before.[4]

Measurements of Osmotic Pressure (with W. N. Broatch).—Osmotic pressures (π) of the amylose acetates in chloroform solution were determined at 22·5° in the instrument previously described.[10] The membrane used was of No. 600 gel-Cellophane, which had been dehydrated by acetone and then conditioned to solvent. The cell constant, for a 0·04 cm. diameter capillary,[11] was independent of the meniscus level, and was reproducible within the setting of the cathetometer (±0·001 cm.). Any deviation from the cell constant which developed after use was thought to be due to contamination of the solution capillaries by evaporated polymer films (particularly with solutions of high viscosity) *although evaporation in this instrument is reduced.* The resultant polymer film is insoluble even after repeated washings of the solution chamber with solvent. Variations in cell constant were not therefore due to (*a*) solute adsorption on the membrane or (*b*) membrane dissymmetry effects. These "effects" observed previously [12] might be similarly explained. Although such variations of cell constant were avoided by working at a different level in the solution capillary, it was considered more satisfactory to dismantle and clean the apparatus.

Concentrations were estimated in duplicate after determination. The procedure adopted was to fill the osmometer with the most concentrated solution, determine the osmotic pressure, remove two volumes (by weight) for estimation of concentration, and add an equivalent volume of solvent. The solution was then carefully mixed, and the osmotic pressure of the diluted solution determined. Pressures were measured statically after an initial setting to 1·0 cm. below the expected value. There was no evidence of solute permeation of the membrane. In each experiment, the value of the intercept $(\pi/c)_0$ was obtained from the linear graph of π/c *versus* c, where π was expressed as dynes g.$^{-1}$ cm., and c as g. cm.$^{-3}$. Number average molecular weights ($\bar{M}_n$) were then calculated from van't Hoff's equation $\bar{M}_n = \boldsymbol{R}T(c/\pi)_0$.

Sedimentation Measurements.—Determinations were made with an electrically driven Spinco ultracentrifuge on both components dissolved in 0·2M-potassium hydroxide. Detailed results will be presented elsewhere.

Results

Examination of Acid-treated Whole Starches.—Microscopic examination showed that the granules which had been treated with acid for 4 hr. appeared unchanged and were still birefringent. However, the granules dispersed more readily to form a less viscous paste. The granules which had been subjected to 24 hr. treatment behaved similarly, although some split granules were visible.

Estimations of the amount of glucose in the acid supernatant liquors showed that after 1 hr. 0·2% and, after a further 3 hr., 0·8% of the granule was solubilised. Hence only about 1% of

[9] I.U.P.A.C. nomenclature, *J. Polymer Sci.*, 1952, **8**, 257.
[10] Gilbert, Greenwood, and Graff-Baker, *ibid.*, 1951, **6**, 585.
[11] Broatch and Greenwood, *ibid.*, 1954, **14**, 593.
[12] Cf. Masson and Melville, *ibid.*, 1949, **4**, 323.

the total weight of the granules was solubilised as glucosans or reducing sugars in the 4 hr. treatment. Chromatographic examination of the gelatinised granules indicated that at the most only traces of oligosaccharides (probably tri- or tetra-glucosans) could have been present after acid-treatment.

An estimate of the apparent reducing power was obtained by titrating the starches directly with the alkaline ferricyanide reagent. The results indicated that the reducing power increased regularly with time of acid-treatment as shown :

Time of acid-treatment (hr.)		0	1	2	3	4
No. ml. of M/100-ceric sulphate required/1 g. of starch	In N_2	0·40	2·80	5·00	7·40	9·83
	In O_2	0·10	4·90	8·75	13·92	18·40

An experiment in the presence of oxygen indicated an apparent greater increase in reducing power.

FIG. 1. (*a*) *Typical iodine-titration curves for acid-treated starches. The original potato starch is shown as the dotted curve. The numbers on each curve are the times of acid-treatment of the starch (in hr.).* (*b*) *Typical iodine-titration curves for acid-treated amylopectins. Potato amylopectin (containing 0·5% of amylose) is shown as the broken curve. Curves 1 and 2 are for amylopectins isolated after 3 and 4 hours' treatment, respectively.*

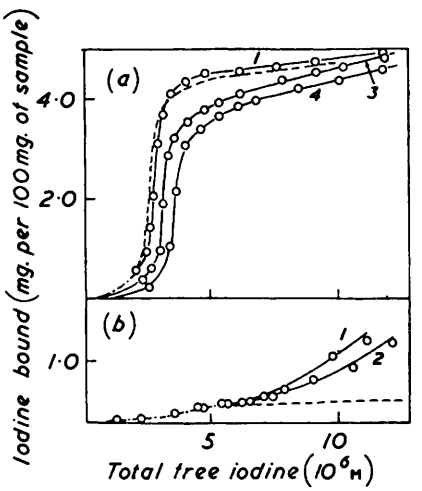
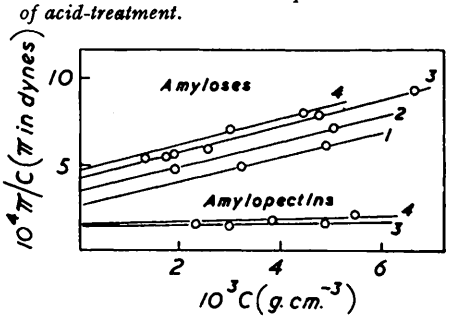


FIG. 2. *Graph of π/c versus c for the acetylated starch components in chloroform solution. The numbers on the curves represent the hours of acid-treatment.*



Potentiometric iodine-titration curves showed changes in iodine affinity of the samples: there was an apparent increase up to about 1 hr. followed by a decrease thereafter (Fig. 1*a* shows typical titration curves). In addition, the slope of the linear portion of the curve increased with time of acid-treatment, as did the activity of free iodine necessary to saturate the linear amylose component.

Time of acid-treatment (hr.)		0	½	1	2	3	4
Redskin starch	Iodine affinity	4·03	—	4·25	—	3·89	3·10
	Amylose (%)	20·70	—	21·80	—	20·00	15·90
Arran Banner starch	Iodine affinity	4·03	4·51	4·30	4·11	3·60	3·07
	Amylose (%)	20·70	23·10	22·00	21·10	18·50	15·80

Properties of the Fractionated Components.—Potentiometric titration showed a normal curve (cf. ref. 4) for the amylose components, whilst the amylopectin curves were abnormal (Fig. 1*b*) and did not show the linear increase in bound iodine previously observed.[5] Fig. 2 shows the results of osmotic-pressure determination for the acetates from Expt. I. The properties of the separated fractions from both experiments are shown in Tables 1 and 2.

The results of physical measurements carried out on solutions of both components in alkali were reproducible, and the solutions were stable for the time required for measurement. The

sedimentation measurements confirmed that depolymerisation effects, particularly in the case of the amylopectin components, were real.

Rates of Degradation.—Degradation of the components was expressed as (1) the number of bonds broken per initial polysaccharide molecule, and (2) the variation of $1/[\eta]$, both as a function of time. The use of the latter function instead of $[\eta]$ directly is justified as follows:

The number of bonds broken per w g. polysaccharide, $(q) = \frac{Nw}{M_0}\left(\frac{M_0}{M_t} - 1\right)$ where M_0, M_t are the number-average molecular weights at times 0 and t, respectively and N is Avogadro's number. This can be rewritten as

$$q = Nw(1/M_t - 1/M_0)$$

$$\text{or} \quad q \propto \frac{1}{M} \propto \frac{1}{[\eta]} \text{ since in this instance } M = K[\eta].$$

Hence the rate of change of $[\eta]$ with time is *not a direct measure of degradation*, in agreement with McBurney.[14]

The graphs of (bonds broken) *versus* t, and of $[\eta]^{-1}$ *versus* t for the two series of amyloses are

TABLE 1. *Properties of the amylose components.*

Redskin amylose

Acid-treatment (hr.)	Iodine affinity	$[\eta]$ in M-KOH	Calc. $\overline{D.P.}$ [a]	$[\eta]$ of acetate [b]	M. wt. from π	Obs. $\overline{D.P.}$ [c]	$10^{13}S_0$ [d]
0	19·5	470	3480	—	—	—	12·0
1	—	220	1630	370	470,000	1630	9·9
2	20·3	190	1370	335	400,000	1390	8·3
3	—	140	1040	305	302,000	1050	7·3
4	20·7	130	925	270	286,000	990	6·2

Arran Banner amylose

Acid-treatment (hr.)	Iodine affinity	$[\eta]$ in M-KOH	Calc. $\overline{D.P.}$ [a]	$10^{13}S$ [e]	Acid-treatment (hr.)	Iodine affinity	$[\eta]$ in M-KOH	Calc. $\overline{D.P.}$ [a]	$10^{13}S$ [e]
0	19·5	520	3850	13·0	3	—	177	1310	—
½	—	280	2070	—	4	—	152	1130	4·8
1	19·3	225	1670	7·1	24	—	73	540	2·9
2	19·8	201	1490	5·8					

[a] Calc.[13] from $\overline{D.P.} = 7{\cdot}4[\eta]$. [b] Measured in $CHCl_3$ solution. [c] Calc. from previous column. [d] Sedimentation constant in c.g.s. units at infinite dilution obtained by graphical extrapolation from $S = f(c)$. [e] Sedimentation constant in c.g.s. units at $c = 0{\cdot}075$ g./100 ml.

TABLE 2. *The properties of the amylopectin components.*

Redskin amylopectin.

Acid-treatment (hr.)	$[\eta]$ in M-KOH	$[\eta]$ of acetate [a]	M. wt. from π	Obs. $\overline{D.P.}$ [b]	β-Amylolysis limit [c]	$10^{13}S_0$
0	182	—	—	—	56·5	175
1	123	220	—	—	57·4	60
2	118	205	—	—	58·2	52
3	113	220	$1{\cdot}1 \times 10^6$	3800	58·1	43
4	105	200	$1{\cdot}0 \times 10^6$	3500	57·5	35

Arran Banner amylopectin.

Acid-treatment (hr.)	$[\eta]$ in M-KOH	Av. length of unit chain	$10^{13}S_0$ [d]	Acid treatment (hr.)	$[\eta]$ in M-KOH	Av. length of unit chain	$10^{13}S_0$ [d]
0	182	25	175	3	108	24	42
½	112	24	63	4	106	24	34
1	122	26	58	24	48	22	14
2	110	25	49				

[a] Measured in $CHCl_3$ solution. [b] Calc. from previous column. [c] Results obtained through the courtesy of Mr. I. D. Fleming and Dr. D. J. Manners. [d] Sedimentation constant in c.g.s. units at infinite dilution obtained by graphical extrapolation from $S = f(c)$.

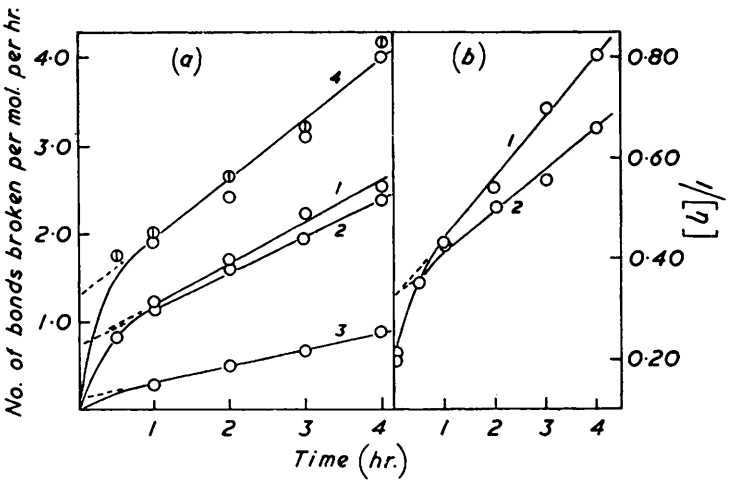
[13] Cowie and Greenwood, unpublished results.

[14] McBurney in Ott and Spurlin's "Cellulose and Cellulose Derivatives," Interscience, New York, 1954, 2nd edn., Vol. I, p. 99.

shown in Fig. 3. It is of interest that in neither instance do the linear portions of the curves extrapolate to the origin. In Fig. 3*a*, the linear portions of the curves correspond to rates of 0·5 and 0·4 (expressed as the number of bonds broken per initial amylose molecule per hr.) for the Redskin and Arran Banner amyloses, respectively. A linear relation (curve 3, Fig. 3*a*) was also found for $(S_0/S_t - 1)$ *versus* t, where S_0, S_t are the sedimentation constants at times 0 and t respectively. The rate of degradation from this curve (0·2) was less than the absolute rate because the diffusion constant for the linear amylose molecule must change considerably with decrease in $\overline{D.P.}$, and hence changes in S were not directly proportional to changes in D.P.

For amylopectins, the degradation could not be calculated unambiguously in view of the difficulty in obtaining absolute values for the molecular weight. However, again it was found that the graph of $(S_0/S_t - 1)$ *versus* t was linear, and on the assumption that for this component

FIG. 3.



(*a*) *Graph showing the number of bonds broken per initial polysaccharide molecule as a function of time.*
(1) *Redskin amylose.* (2) *Arran Banner amylose.* (3) $(S_0/S - 1)$ *for Redskin amylose.*
(4) $(S_0/S - 1)$ *for Redskin* (○) *and Arran Banner* (ⓘ) *amylopectins, respectively.*

(*b*) *Graph of* $1/[\eta]$ versus t *for the amylose components.*
(1) *Redskin amylose.* (2) *Arran Banner amylose.*

the diffusion constant was unlikely to vary considerably, the linear portion of this curve corresponded to a rate of 0·7 (units as above). This is likely to be a minimum rate as any disproportionate increase in the diffusion constant with time of degradation will increase the slope. Again the extrapolated linear portion of this curve did not pass through the origin.

DISCUSSION

Experimental conditions were chosen such that our results are strictly comparable with those of both Meyer and Menzi [2] and of Kerr.[3] However, these authors presented insufficient data to permit comparison of actual *rates* of degradation of the two components. (Kerr's data unfortunately could not be replotted since " fluidities " were reported rather than times of acid-treatment.) At best, only general comparisons are possible.

The starches from the two varieties of potato (Redskin and Arran Banner) gave similar results, and hence can be considered to behave identically.

General Properties of Acid-modified Granules.—The action of acid does not alter the birefringent properties of the granule. There was no evidence of swelling in the acid media. It therefore appears that acid-treatment has preferentially affected the *amorphous* rather than the crystalline region of the granule. Sjoström [15] and Meyer and Bernfeld [16] observed similar effects, and Alsberg [17] also reported the treatment with hydrochloric

[15] Sjoström, *Ind. Eng. Chem.*, 1936, **28**, 63.
[16] Meyer and Bernfeld, *Helv. Chim. Acta*, 1940, **23**, 890.
[17] Alsberg, *Plant Physiol.*, 1938, **13**, 295.

acid (7·5%) destroyed the ability of the granule to swell and suggested that the portion responsible for swelling had been modified.

It was thought that estimation of the amount of reducing sugar produced in the acid supernatant liquor was the only suitable method for determining any loss in weight of the granule on acid-treatment. Only 1% was found after 4 hr. The possibility that oligosaccharides might have been produced and that these were unable to diffuse out readily from the persistent granular structure and so account for (1) the abnormal iodine titration curves for the whole starches and the amylopectins, and (2) the β-amylolysis results (particularly as the amylopectins were freeze-dried directly) was shown to be incorrect.

Potentiometric titrations indicated changes in the apparent percentage of linear material in the granule. These results can be explained by assuming that the material dissolving in the first hour is primarily amylopectin (the 0·2% of granular weight lost accounting for a rise of 0·3—0·5% in the effective amylose-content), whilst that dissolving later is primarily amylose (the further 0·8% of granular weight lost being equivalent to about 4% of total amylose content).

Although Meyer and Menzi [2] reported no change in iodine-binding power of their acid-treated starches, it is of interest that the acid-modified maize starches studied by Schoch and his colleagues [18] also possessed iodine affinities very much lower than the original. The iodine affinity of starch can only be unaffected if the degradation products are solubilised completely (and there is no evidence for this), or acid-modified components have unaltered iodine-binding characteristics. However, although the fractionated amyloses possessed the usual iodine binding characteristics, all the amylopectin fractions bound more iodine than normal. The relative constancy of length of unit chain and β-amylolysis limits suggests that purely random hydrolytic action must have occurred, and no explanation can be advanced for this behaviour. The increased slope of the linear portion of the titration curves for the whole starches is probably accounted for on this basis.

The method of isolation of the amylose involved reprecipitations as the butan-1-ol complex which might involve the loss of some short chains. This was decreased in the case of Arran Banner samples by carrying out only one recrystallisation, although in neither experiment was there any apparent loss in amylose at this stage.

Degradation of the Components in the Granule.—The curves of rates of degradation (Fig. 3*a*) show that amylopectin is degraded considerably faster than amylose. Since the former must be a minimum rate, and also since the α-1 : 6-bonds (present to the extent of about 1 in 20) are reported [1] to be stronger than α-1 : 4-bonds, amylopectin must be preferentially degraded and is therefore far more accessible to attack than the amylose. The curves for both components do not extrapolate to zero time, which indicates (by comparison with cellulose chemistry) that acid-modification of the granule takes place in two stages, a rapid attack on amorphous regions, followed by a slower attack on more crystalline areas. This occurs for both components, but the results indicate a larger preferential scission of amylopectin rather than of amylose initially. (Although this two-stage process might well indicate the preferential scission of anomalous linkages, it is thought that in experiments involving whole starch granules such interpretations have to be made with caution.)

The results show that, even after 24 hr., degradation of amylose in the granule is relatively limited (to about 6 bonds per initial molecule). This contrasts markedly with the behaviour of the isolated amylose under similar conditions (preliminary unpublished experiments having indicated that such degradation is much more rapid), and suggests in contradiction to Lathe and Ruthven's conclusions [19] that penetration into the inner region of the *unswollen* granule is time-dependent and not instantaneous. Any *initial*

[18] Lansky, Kooi, and Schoch, *J. Amer. Chem. Soc.*, 1949, **71**, 4066.
[19] Lathe and Ruthven, *Biochem. J.*, 1956, **62**, 665.

attack will therefore occur preferentially in the surface layer of the granule before hydrogen ions diffuse into the inner regions.

The different rate curves for the two amylose fractions could be due to the prior storage of Redskin starch in methanol with subsequent dehydration and " splitting " of the granular structure permitting more ready access of reagent. This effect is not thought to be significant in these experiments.

Our degradation results appear to support Kerr's conclusions [3] that amylopectin is preferentially degraded. Ulmann [20] has reached a similar conclusion from chromatographic evidence. In view of the relatively small changes in the number of bonds broken, the changes in reducing power observed by Meyer and Menzi [2] can probably be explained by the " peeling action " [21] of their reagent.

Granular Structure.—Little is yet definitely known,[1] although crystallinity is evident in all starch granules (including those of the waxy variety). The formation of " crystallites " by the alignment of the long unbranched amylose molecules is very likely, but in potato starch granules amylose accounts for only 20% of the structure. The question of the compacting of the remaining 80% is far more difficult, even to account for the reported *total* 50—60% crystallinity of the granule.[22] In particular, it is difficult to see how amylopectin molecules can pack themselves to any degree of crystallinity if they have the three-dimensional structure envisaged by Meyer. As suggested by Greenwood,[1] a two-dimensional structure might be more likely. Much depends on the actual shape of the amylopectin molecule, and experiments are in progress to determine this.

Unless amylose exists preferentially at the surface (no evidence exists for this), the surface layer of the granule must be predominantly amylopectin by virtue of its amount. (Evidence for the existence of a true amylopectin outer layer is contradictory, but unpublished experiments have indicated that such a layer may exist.) The amylopectin is most likely to form the amorphous regions of the granule, although, as Meyer has suggested, the outer branches of several amylopectin molecules might well align themselves to form a small crystallite. In addition, the outer branches might well link up with amylose in the crystalline regions, and amylose (particularly the short-chain material) might well form part of the amorphous region. Swelling of the granule would be associated with the amorphous branched component.

This concept of granular structure is consistent with the effects observed on acid-treatment. The primary attack of acid on the potato granule is on the surface membrane, because of the very limited swelling at the temperature of the experiment. The more freely accessible outer chains of the surface amylopectin molecules might well be degraded sufficiently to dissolve completely. This occurs mainly in the first hour, and is accompanied by a simultaneous attack on amylose and amylopectin in the amorphous regions. Degradation of the components arises by diffusion of the acid into the granule in the absence of swelling, and degradation takes place throughout the granule, but at a decreased rate in the crystalline regions. The hydrogen bonding responsible for the crystalline structure must be relatively strong by virtue of the limited hydrolytic effects observed even after 24 hours' treatment.

In view of the complex, but ordered, nature of the structure of the granule, it is not surprising that the mode of action of the acid is complex. Further methods of studying granular structure are being examined.

The authors thank Professor E. L. Hirst, F.R.S., for his interest in this work, and the Rockefeller Foundation for financial assistance.

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