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RETROGRADATION KINETICS OF WAXY-CORN AND POTATO STARCHES; A RAPID, RAMAN-SPECTROSCOPIC STUDY*

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ABSTRACT

The retrogradation kinetics for waxy-corn starch–water and potato starch–water systems were monitored by rapid-scanning, Raman spectroscopy. The spectra showed that bands in the 480-cm^{-1} skeletal-mode region and the 2900-cm^{-1} C–H stretching-mode region are sensitive to the retrogradation process. Kinetic data obtained from the time dependence of these modes show a multi-stage process. This process is discussed in terms of the formation of local and long-range order in the starch polymers.

INTRODUCTION

Native-starch granules are insoluble in cold water. On heating starch–water mixtures, granule swelling, hydration, and solubilization occur. This process, gelatinization, involves melting of the crystallites that are present in the native granules. On cooling gelatinized starch-pastes, gel formation, turbidity, and syneresis of water may occur. This phenomenon is termed retrogradation. For starch-containing food products, gelatinization followed by retrogradation is a major factor in determining product-texture. An example is the staling of baked goods, which has been correlated with the retrogradation of the starch. As a consequence, the time course and kinetics of the retrogradation process are important technologically, and many studies of them have been undertaken. Nonetheless, the kinetics and mechanism, particularly at the molecular level, are not yet well understood.

The kinetics of amylose and starch retrogradation have been monitored by thermal analysis[1], light scattering[2], digestibility with enzymes[3], iodine binding[4], quantitative separation of the retrograded form by centrifugation[5], X-ray

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diffraction[6,7], and a variety of rheological techniques[8–12]. However, these studies invariably involved long time-scales of many hours and days, and none gave information on the processes involved at the molecular level. Even the X-ray diffraction technique is limited to the long-term appearance of the final, crystalline form of the retrograded material.

We now present a new approach to this problem, using rapid-scanning Raman spectroscopy to monitor the retrogradation of potato starch–water and waxy-corn starch–water gels in a quasi-continuous manner. This method allows polymer crystallization processes to be monitored at the molecular level, with a time resolution of ~15 s. The data indicate that the retrogradation process for these starch–water gels occurs in four distinct stages, each of which may be characterized by its own kinetics. Information about the molecular mechanisms which lead to these multi-stage kinetics, while not completely clear, can also be gleaned from the data.

Good-quality Raman spectra and interpretation had been reported for powder samples of V-amylose and B-amylose, and indicated that this technique is sensitive to the conformational differences between these two polymers[13,14]. There are no reports on the Raman spectroscopy of amylopectin, although a preliminary investigation of the more highly branched material glycogen, as a dry powder, has appeared[15]. Only preliminary investigations on the Raman spectroscopy of native starch granules have been reported[15–17]. Although the presence of water presents no problems in Raman spectroscopy, there have been no reports of Raman spectra for starch–water systems.

EXPERIMENTAL

Raman spectra were recorded with a Jobin-Yvon HG2S Raman spectrometer modified for rapid scanning as described previously[18]. Excitation was produced by an argon ion laser (488 nm) operated at 250 mW output power.

All starch samples were provided by National Starch and Chemical Corporation. Amylose and amylopectin were both from potato. The potato amylose was prepared by National Starch, and the amylopectin was obtained from Avebe. Samples for Raman spectroscopy were prepared in 1-mm, glass capillary tubes. For examination of starch–water gels, this involved using weighed amounts of the starch (52%) and water (48%), sealing the capillary, and heating for 20 min in a bath at 90° in order to gelatinize the starch. Spectra were recorded at 90°, and then the samples were cooled to room temperature. The retrogradation process could be monitored continuously by using rapid-scanning Raman spectroscopy.

Wide-angle, X-ray diffraction patterns were measured by using a Philips powder diffractometer (PW 1050/1390) mounted on a PW 1730/10 sealed-tube, X-ray generator operated at the CuKα wavelength of 1.542 Å. Measurements of diffracted intensity were made over the angular range of 2 to 20°. The temperature of the samples was 20°.

Small-angle X-ray scattering was carried out by using a Kratky slit-focussed SAXS camera mounted on a Philips sealed-tube X-ray generator as before. Scattered X-ray intensities produced by the pregelatinized sample of close-packed waxy-corn starch–water in capillaries were collected by a linear, position-sensitive, proportional X-ray detector, and transferred for processing to a Canberra multichannel analyzer and DEC MINC computer. The data set was corrected by subtracting capillary-holder and aqueous-solvent background contributions.

RESULTS AND DISCUSSION

Spectra of starch and component powders. Fig. 1 shows the Raman spectra, in the 1600- to 200-cm^{-1} range of powders of amylose, amylopectin, potato starch, and waxy-corn starch. Fig. 2 shows the spectra of the same four samples in the C–H stretching region, 3100—2800 cm^{-1}. A cursory examination of these Figures indicates that all samples give very similar spectra, and this was to be expected. There are no bands that can be clearly identified with the branch points in the amylopectin.

There are, however, a number of small, but significant, differences between these spectra. For example, Fig. 2 shows that there are relative-intensity differences between the major peaks in the C–H stretching region for the different samples. Although this is a complex of bands in each case, a measurement of the overall half band-width (full width at half maximum) for the band envelope provides a measure of the frequency and intensity variations that occur. The half-width also changes

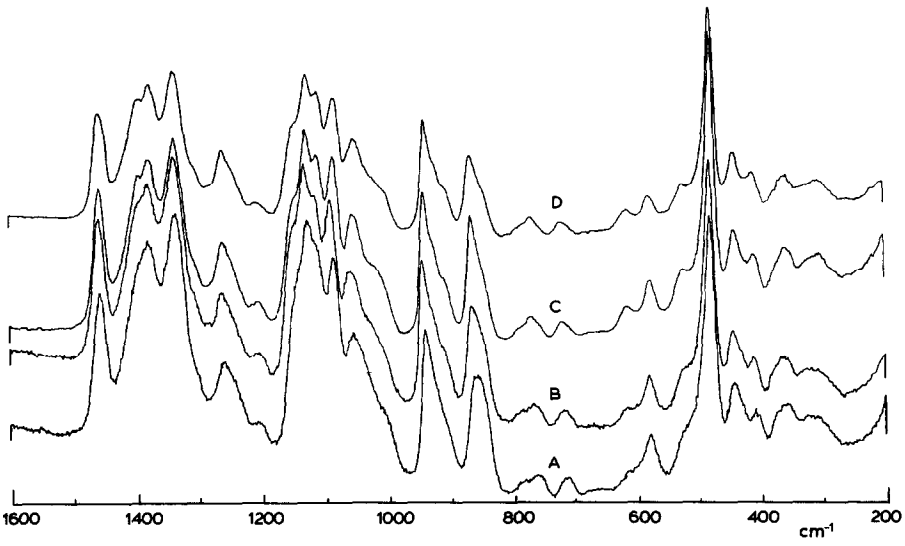

Fig. 1 Raman spectra of (A) amylose, (B) amylopectin, (C) potato starch, and (D) waxy-corn starch powders (1600–200 cm^{-1})

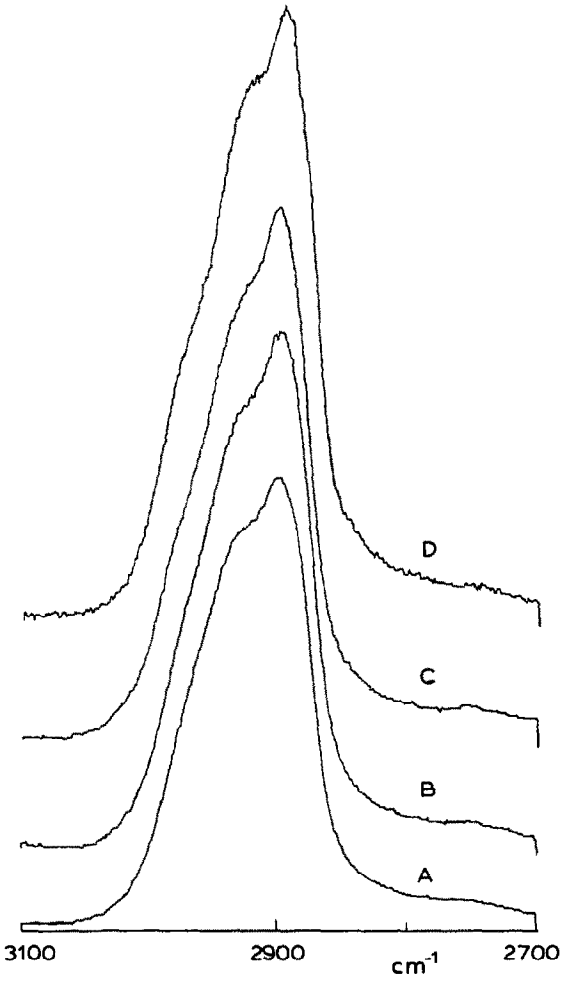

Fig. 2. Raman spectra of (A) amylose, (B) amylopectin, (C) potato starch, and (D) waxy-corn starch powders (C–H stretching region).

for the intense, but more isolated, mode at 480 cm^{-1}, which is assigned as a skeletal mode. These data, as well as others to be presented, are summarized in Table I.

Other differences are also seen. These are in the spectral regions near 1380, 1125, 615, and 520 cm^{-1}. All of these changes are of the same type, that is, bands, or splittings of bands, appear more distinctly in the starch spectra than in those of the isolated polymers (amylose and amylopectin). This observation is consistent with the fact that the starches used here are known to be more crystalline than our samples of the isolated polymers. Such splittings and sharpening of bands would be expected for the more crystalline materials.

Waxy corn starch–water spectra. Samples containing 52% of starch and 48% of water by weight were prepared and studied. At this composition, there is no separation of free water. This composition is referred to as close-packed.

TABLE I

HALF BAND-WIDTH IN RAMAN SPECTRA OF STARCHES

Sample	*Wavenumber and half-widths (cm^{-1})*[a]	
Amylose powder	480	2900
	26	99
Amylopectin powder	480	2902
	25	98
Waxy-corn starch powder	477	2903
	18	91
Close-packed, waxy-corn starch at room temperature	475	2904
	18	84
Waxy-corn starch plus of 50% water, at 90°	482	2906
	29	105
Cooled immediately	481	2905
	26	96
Aged 1 day	477	2901
	18 5	82
Aged 1 week	475	2902
	18	80
Aged 4 weeks	475	2901
	17 5	80

[a]The top two figures for each sample are the wavenumbers and immediately below are the respective half-widths

Fig. 3a shows the Raman spectrum of this system upon mixing at room temperature. On comparing this spectrum with that of the powdered waxy-corn starch, it was found that the changes associated with crystallinity continue to emerge when water is added. This is consistent with the observations of others that

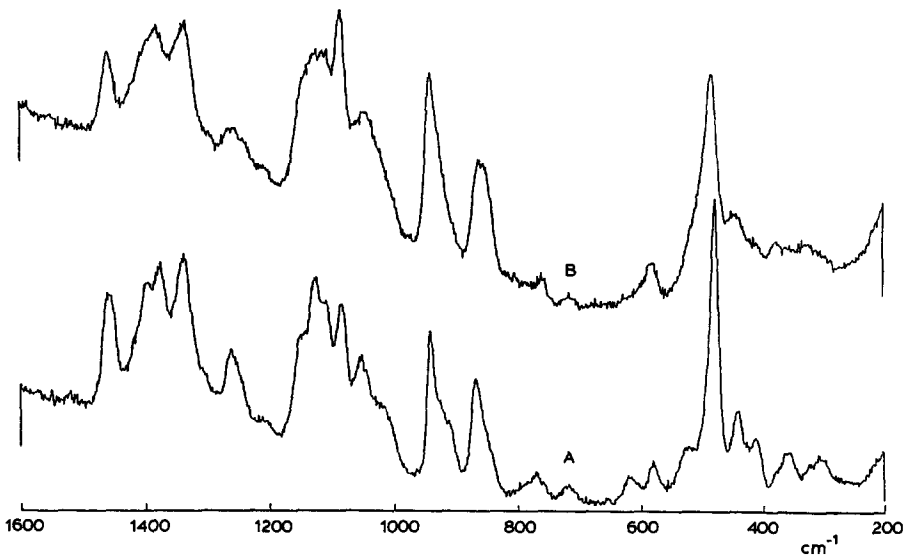

Fig 3 Raman spectra of waxy-corn starch–water, close-packed system A, room temperature, prior to gelatinization; B, heated to 90°.

addition of water to starch powder increases the crystallinity. This is also observed in the C–H stretching region (not shown).

When this sample is gelatinized by heating to 90° in a sealed tube, the opaque sample clears completely, and a solution is formed. The Raman spectrum at 90° is shown in Fig. 3b. Many changes occur in this process. The intense band at 475 cm^{-1} (at room temperature) shifts to 482 cm^{-1}, and its half width increases from 19 to 29 cm^{-1}. In the C–H stretching region, the half band-width of the band envelope increases from 84 to 105 cm^{-1}. Other changes occur in the 1380-, 1125-, and 615-cm^{-1} regions. Even allowing for spectroscopic changes resulting from the 65° difference in temperature, it is clear that changes have taken place in the starch sample which are manifested in the Raman spectrum.

This point was made clear by the following experiment. A sample was heated from room temperature to 65°, a temperature at which gelatinization will not occur. The spectrum of this sample was compared with that of a sample heated to 90° and then cooled to 65°. In this way, spectra of the two different phases are obtained at the same temperature. In this case, there remains a 4-cm^{-1} difference in wavenumber maximum, a 5-cm^{-1} difference in half width for the 475-cm^{-1} band, and an 8-cm^{-1} difference in half width in the C–H stretching region. Although the differences are lessened, they remain substantial.

To study the retrogradation of a waxy-corn starch–water gel, a sample that

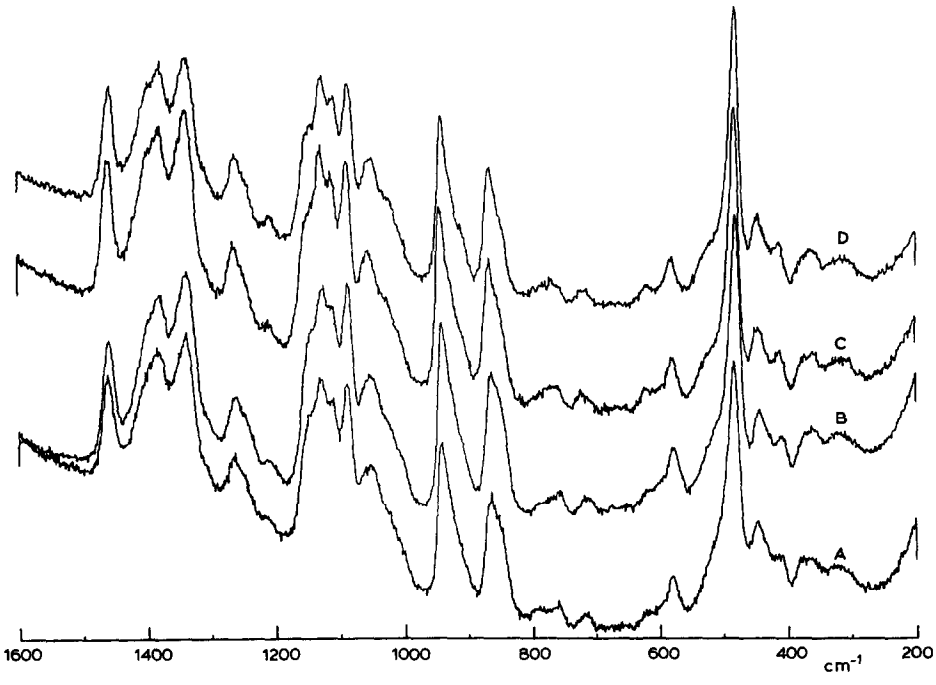

Fig. 4 Raman spectra of waxy-corn starch–water, close-packed system, at room temperature, A, immediately after cooling from 90°, and after aging for B, 8 h, C, 24 h, and D, 48 h

had been heated to 90° was cooled to room temperature, and the Raman spectrum recorded immediately. This spectrum, shown in Fig. 4, has many of the features of the spectrum of the hot sample. In particular, frequency shifts and increased half widths, already described, remain for the cooled sample. Aging of this sample for one day at room temperature results in a spectrum that is similar to the original, room temperature spectrum. Further changes continue to occur, including band narrowing and shifts in frequency, over several weeks. These data are summarized for the C–H stretching and skeletal mode near 480 cm^{-1} in Table I, and may be seen in Fig. 4, which shows the spectrum of this sample after aging for 8, 24, and 48 h

Waxy-corn starch–water retrogradation kinetics. The data just presented indicate that, in the Raman spectrum, there are changes which are reporting on the time-dependent processes occurring during retrogradation of waxy-corn starch–water gels. We now turn to results of the study of kinetics, using these changes to monitor the process.

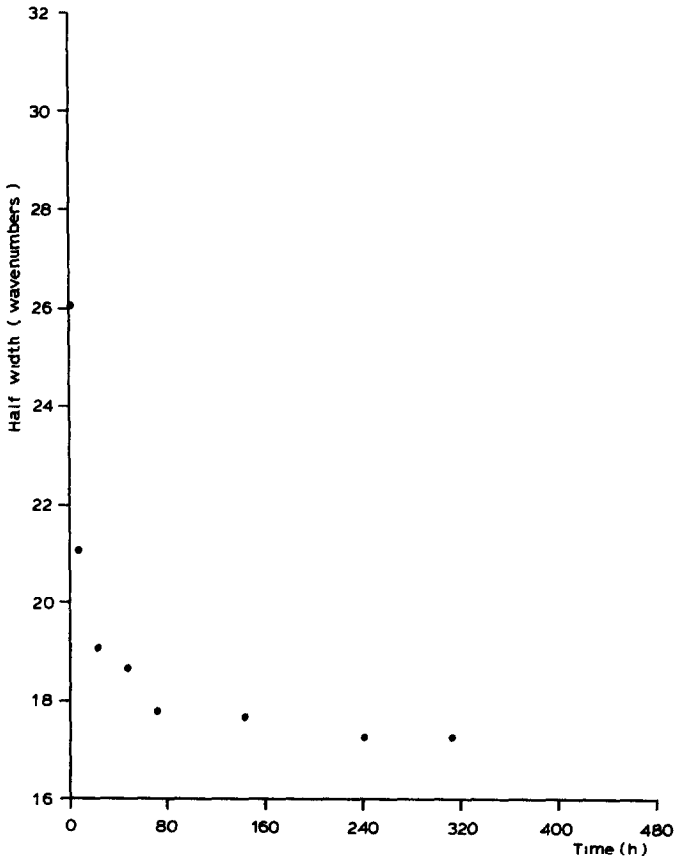

Fig 5. Half width of the 480-cm^{-1} band in the Raman spectra of the waxy-corn starch–water system with time, sampled at long intervals over a two-week period.

Although many parameters from the spectra could be used, we selected two measurements for monitoring the kinetics. These are the half band-widths of the band near 480 cm^{-1} and of the C–H stretching-region envelope. It should be clear that these parameters are to some extent arbitrary, but they carry information on the vibrational modes of the part of the molecules that give rise to them. Because they are half band-width measurements, they need not be proportional to concentration. However, in other studies of polymer crystallinity, we showed[18] that the half band-widths of certain Raman bands often relate directly to such properties as density in semicrystalline polymers, and thus to the degree of crystallinity of the sample. Moreover, half band-widths are useful for monitoring kinetics by Raman spectroscopy, because they are true spectroscopic parameters, unaffected by the amount of sample in the scattering volume, the sample-cell geometry, and other experimental variables. This is not true for peak intensities.

The first goal of these kinetic measurements was to compare results from Raman spectroscopy with those from other techniques. To do this, data were

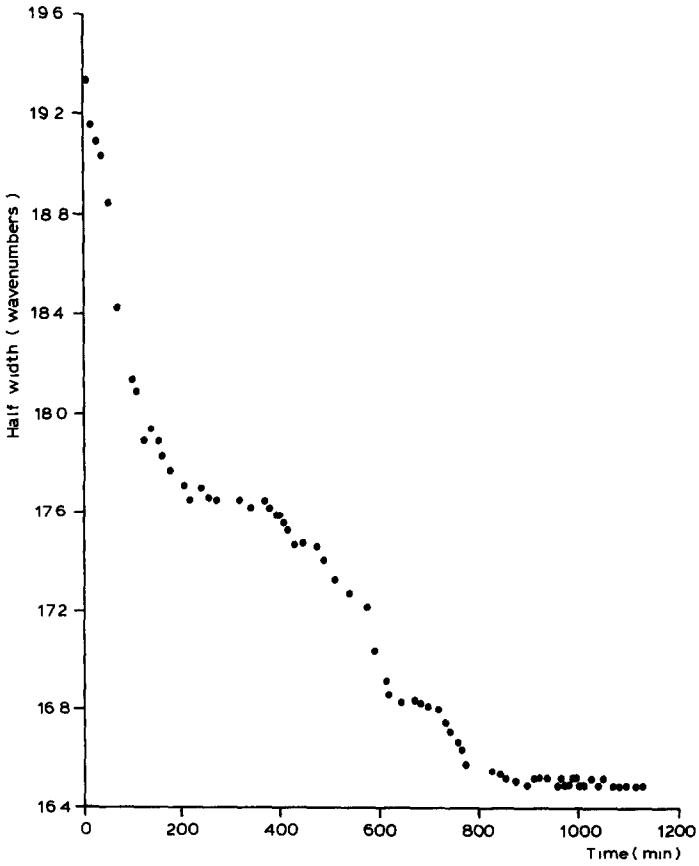

Fig 6 Half width of the 480-cm^{-1} band in the Raman spectra of the waxy-corn starch–water system with time, measured by using rapid-scanning Raman spectroscopy

obtained at long intervals over several weeks. The data for the 480-cm^{-1} band are shown in Fig. 5. They appear to show a smooth, nearly exponential, narrowing of the half width over this period. A very different picture of the kinetics is obtained, however, when the process is monitored by rapid-scanning Raman spectroscopy over the first 18 h. These results are shown, for the 480-cm^{-1} and C–H stretching-region half-widths, in Figs. 6 and 7. Instead of the smooth decay, this increased time resolution shows that the process actually occurs in four stages.

Stage 1 begins immediately after cooling, and consists of a rather rapid change over the first 3 h. At this point, the process stops, and a plateau region, Stage 2, is observed. This region lasts for ~4 h, at which point, change resumes (Stage 3), but with kinetics rather different from those observed in Stage 1. This much slower process lasts for ~6 h in the case of the 480-cm^{-1} band, and 9 h for the C–H stretching modes. This stage is the first one for which the two spectroscopic regions show significantly different rate-constants. Finally, there is a long, slow

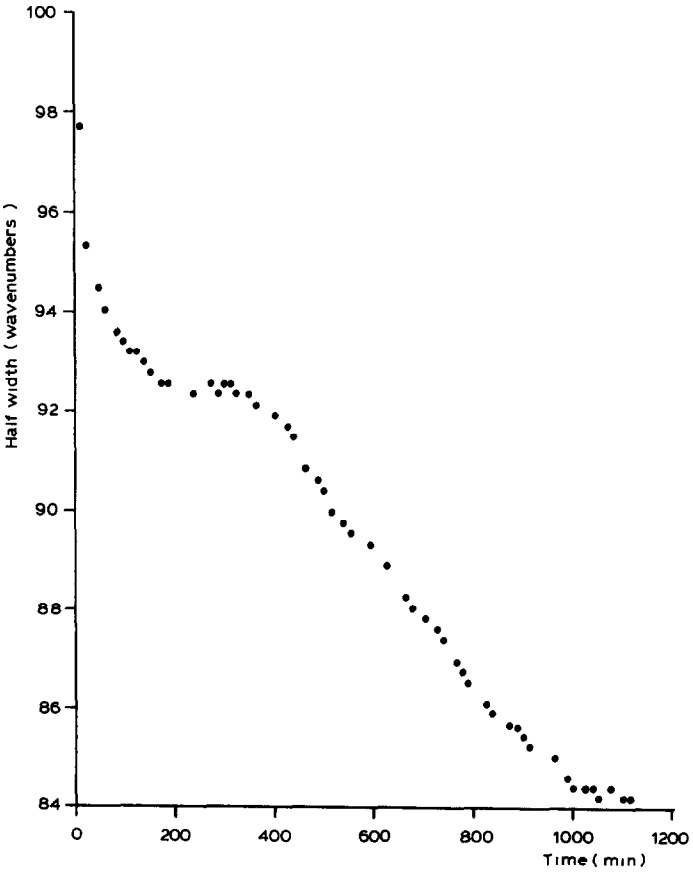

Fig 7 Rapid-scanning Raman results for the C–H stretching region in the spectrum of the waxy-corn starch–water system

change, much smaller in magnitude (Stage 4), which occurs over several weeks. These data are not shown in Figs. 6 and 7, but have already been given in Fig. 5. Thus, the rapid-Raman data show that the retrogradation kinetics is much more complex than that found by sampling at long intervals.

Potato starch–water spectra. Fig. 8 shows the Raman spectrum of a sample prepared by mixing 52% of potato starch with 48% of water, gelatinizing at 90°, and cooling to room temperature. As in the case of waxy-corn starch, the addition of water to potato-starch powder results in spectroscopic changes indicative of increased crystallinity, such as narrowing of the half band-width of the 479-cm^{-1} band.

Aging of this sample was carried out as described for waxy-corn starch, and with similar results However, in this case, retrogradation is much more rapid. After only 6 h, the spectrum obtained is very similar to that of the initial sample, and by 50 h, changes measurable by Raman spectroscopy have ceased. This more rapid process is again consistent with literature observation on potato starch. These results are seen in Fig. 8, and are summarized in Table II.

Potato starch–water retrogradation kinetics. Using the same spectroscopic parameters as for waxy-corn starch, it is possible to monitor the retrogradation kinetics of potato starch–water gels

Fig. 9 shows the results of measurements taken at intervals of ~1.5 h. These may be contrasted with the analogous experiment for waxy-corn starch, shown in

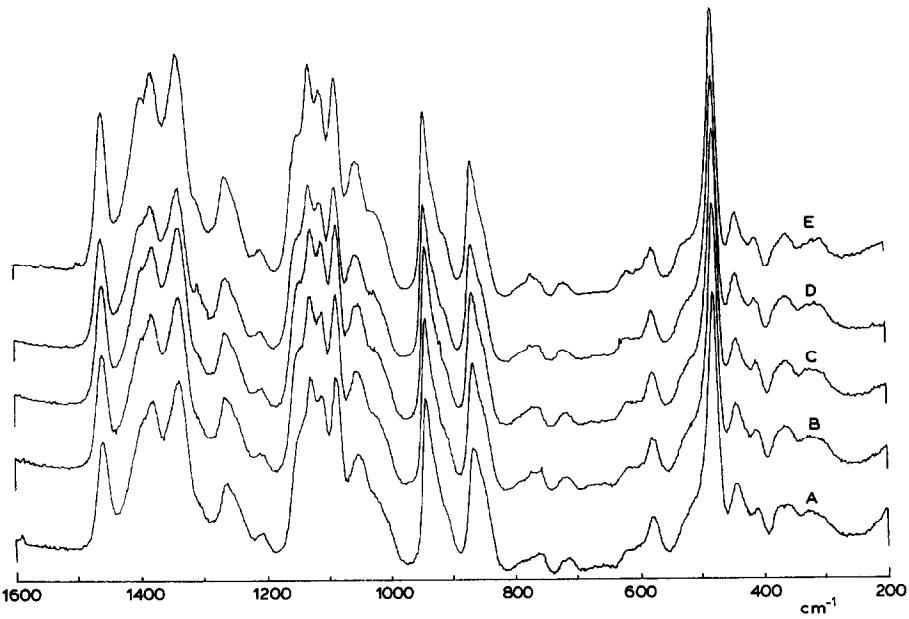

Fig 8 Raman spectra of the potato starch–water, close packed system at room temperature A, Immediately after cooling from 90°; and after aging for B, 1 5 h, C, 3 h, D, 6 5 h; and E, 50 h

TABLE II

CHANGES IN RAMAN SPECTRA OF POTATO STARCH–WATER CAUSED BY VARIOUS TREATMENTS

Sample	*Wavenumber and half-widths (cm^{-1})*[a]	
Potato-starch powder	477	2803
	17 62	88.76
Close-packed, potato starch at room temperature	475	2903
	16 95	78.82
Close-packed potato starch at 90°	481	2906
	24 17	95 95
Cooled to room temperature, immediately	481	2905
	22 44	89 17
Aged 1 day	479	2905
	17 43	83 93
Aged 6 days	479	2904
	17 26	83 02
Cooled to 0°, immediately	477	2902
	18 90	86 94
Cooled to 0°, aged 1 day	476 5	2901
	17 54	83 45

[a]The top two figures for each sample are the wavenumbers and immediately below are the respective half-widths

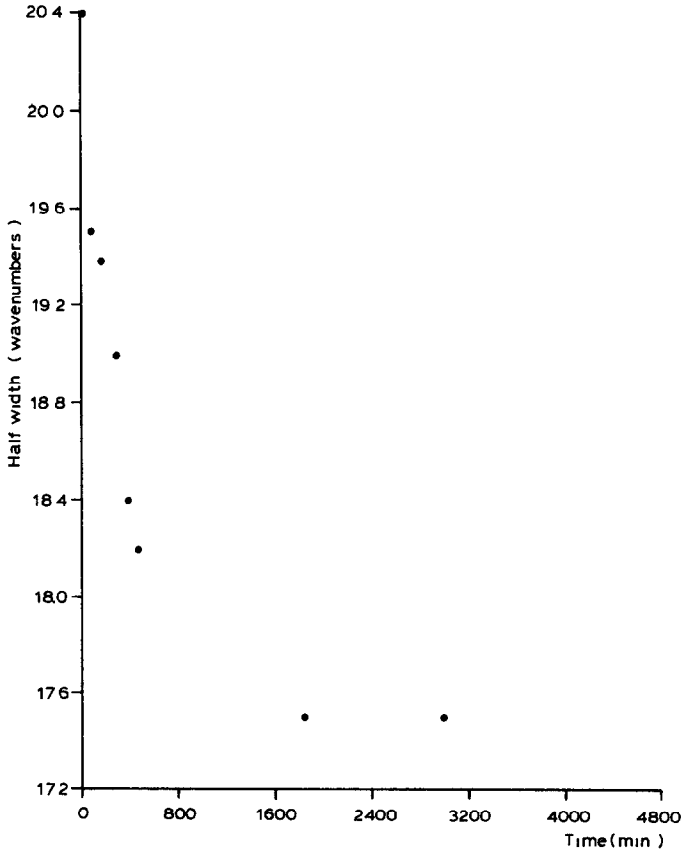

Fig 9 Half width of the 480-cm^{-1} band in the Raman spectra of the potato starch–water system sampled at long intervals over three days

Fig. 5. Although the apparent kinetics are similar, the rate is much higher for potato starch.

As in the case of waxy-corn starch, a very different picture emerges when the time resolution of the measurements is increased. This is shown in Figs. 10 and 11 for the 480-cm^{-1} and C–H stretching regions. Once again, the four-stage process is observed, and every stage is faster than for waxy-corn starch.

Retrogradation of waxy-corn starch by X-ray diffraction. To aid in elucidating the origins of these stages, we examined the retrogradation process by X-ray diffraction for the waxy-corn starch system.

The results of both wide-angle X-ray diffraction and small-angle X-ray scattering measurements are in agreement, and indicate the absence of crystallization at times <8 h (*i.e.*, before the start of Stage 3). Subsequently, there is evidence from both techniques for the slow formation of crystalline regions. Both X-ray techniques monitored the onset of crystallization by examining the development of

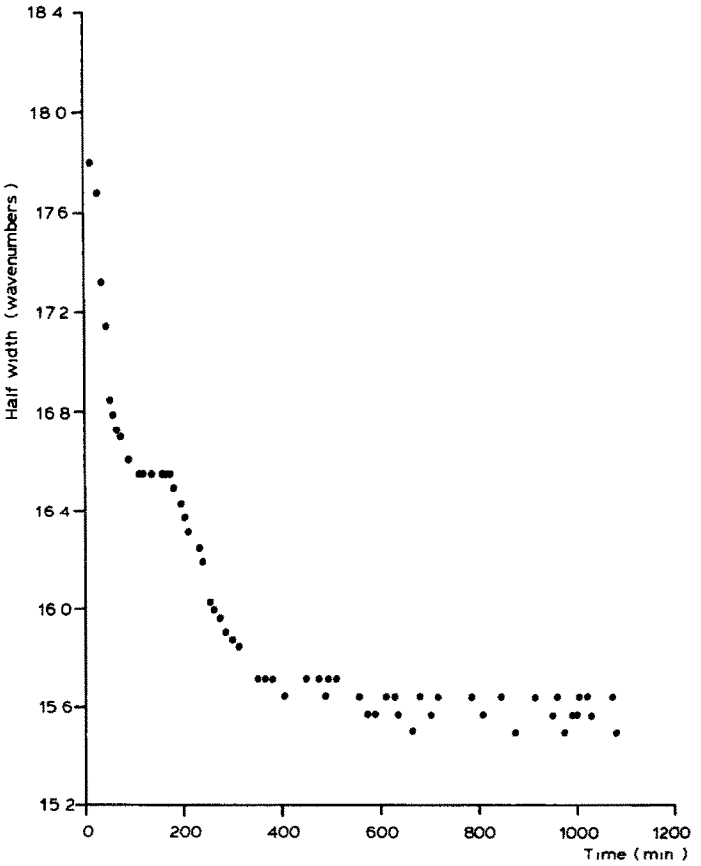

Fig 10. Half width of the 480-cm^{-1} band in the Raman spectra of the potato starch–water system with time, measured by using rapid-scanning Raman spectroscopy.

the 100 reflection (*d* spacing, 1.6 nm) characteristic of starch B-type crystal-structure. The small-angle X-ray-scattering data were collected during 100 h after gelatinization and cooling, and the crystallinity continued to increase throughout this period.

Using small-angle X-ray scattering, we also noticed, at the smaller angles, very substantial changes occurring concurrently with the crystallization process. The exact origins of the changes have yet to be identified, but they are clearly associated with the ordering, aggregation, and crystallization of starch. Similar effects had been reported for starch derivatives[19,20].

Kinetic analysis. The results indicate that, for both waxy-corn and potato starch, addition of water, known to increase crystallinity as measured by X-ray diffraction, results in several small, spectroscopic changes. These are of the type expected for increased crystallinity in a polymer, namely, sharpening of several bands in the spectrum. Even in those regions where it appears that there are splittings of bands, it is unlikely that both components exist in broader envelopes

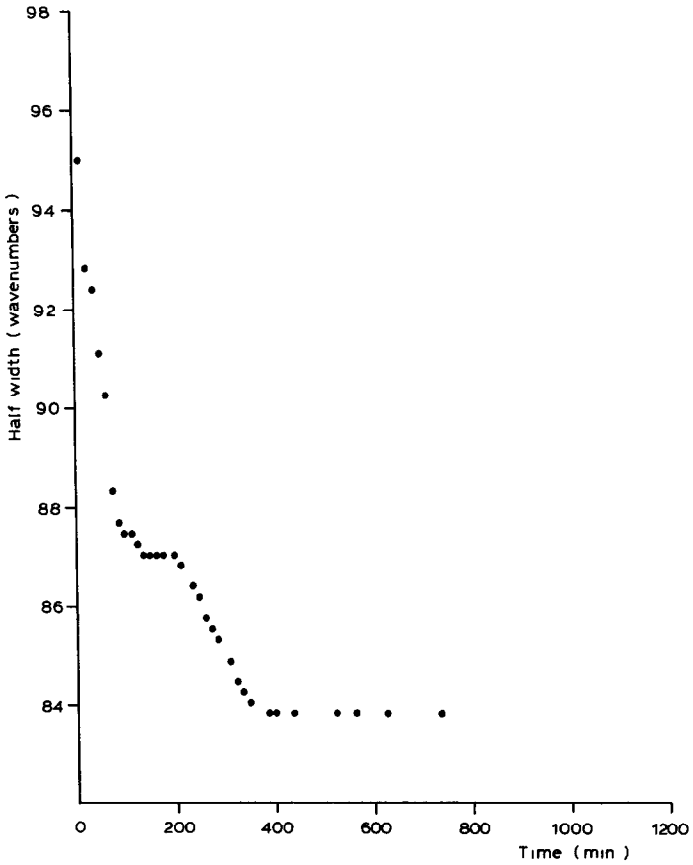

Fig 11 Rapid-scanning Raman results in the C–H stretching region for the potato starch–water system

in the less-crystalline material, and appear only as distinct maxima as half-widths decrease.

All of the kinetic data obtained by us used the C–H stretching region and the "skeletal mode" near 480 cm^{-1}. The skeletal mode, as described by Cael *et al.*[13,14], is a strong, fairly symmetrical band. Further insight into its origins has come in a separate study reported by us[21]. This band does not appear in the spectrum of D-glucose or maltose, and becomes progressively more intense in the spectra of malto-oligomers as the degree of polymerization (d.p.) increases, up to a d p. of ~60, where no further increase is observed for powders. In fact, however, this apparent increase in intensity is due to a narrowing of the band as the d.p increases. It should also be noted that, although the Raman spectrum of cellulose is very similar to that of starch, this band does not appear in the cellulose spectrum. This explains why such a band might undergo a marked change in half width when the starch–water systems are heated to 90°.

The envelope in the C–H stretching region is complex, containing many bands. These arise from the different C–H bonds, from symmetric and asymmetric modes of the methylene group, and from Fermi resonance between the overtone of the CH_2 deformation and stretching fundamentals. Although it was once thought that C–H stretching modes are rather insensitive to conformational and environmental changes in polymers, it is now recognized that this is not the case. In polyethylene, in many lipid–water gels, and in other cases, it has been shown that the C–H stretching region is affected by conformational change and by packing of chains. Thus, it is not surprising that this should be observed here, although the complexity of the band envelope makes it difficult to determine which components are responsible for the changes observed.

We now turn to the kinetics observed. Previous investigations of the kinetics of starch retrogradation, bread staling, and related phenomena have almost all used the Avrami equation to characterize the changes. In this approach, well summarized and much criticized in the literature[22], the fractional crystallization Θ (normalized to a 0 to 1 scale) is expressed in terms of a rate constant k and Avrami exponent n by the equation $\Theta = \exp(-kt^n)$.

Most criticism of the use of the Avrami equation has been of the interpretation of the exponent n in terms of the nature of the crystallization process. However, it may also be criticized as being too sensitive to the shape of the initial stage of the transition and as being the imposition of a model on the results.

Nonetheless, the value of the Avrami equation, where it is used carefully, is to provide a basis for comparing kinetic results between different techniques. We shall use it for this purpose

As shown in Fig. 5 and 9, when the retrogradation of either waxy-corn or potato starch is sampled at long intervals over a period of many days or weeks, an apparent smooth change in the spectroscopic parameters is observed. Because our multistage kinetics (see Figs. 6, 7, 10, and 11) are so different from anything that has previously been measured for starches, it seemed desirable to confirm that,

when the Raman measurements are carried out at long sampling-intervals, they give results similar to other techniques, *i.e.*, that the difference is only one of sampling interval. To do this, the results in Figs. 5 and 8 were reduced according to the Avrami equation. The Avrami plots obtained, illustrated in Fig. 12 for the potato starch system, yield n values between 0.5 and 1.0, comparable to those previously reported. Clearly, however, in light of the higher time resolution data to be discussed now, these results conceal the actual events occurring in the first time-period.

In treating the multistage kinetic processes shown in Figures 6, 7, 10, and 11, it is not sensible to use the Avrami equation. The goal in reducing these kinetic data is to provide a basis for comparing the two starches, as well as the two spectroscopic regions, and to set up a framework for more-extensive kinetic measurements on these and other systems. To accomplish this, it is proposed to treat Stage one as first-order kinetics, which it closely approximates. To illustrate the suitability of this approach, Fig. 13 shows a first-order kinetic plot for Stage 1 of the C–H region of the waxy-corn starch system. These results are typical of the four cases. Stage 2,

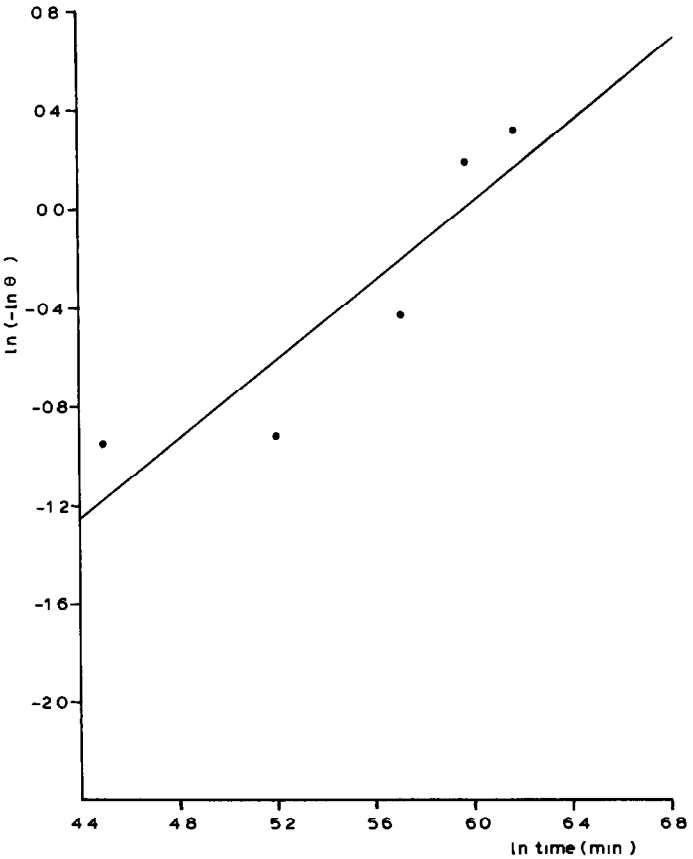

Fig 12. Avrami plot made from the data of Fig. 9

the plateau, can be characterized simply as a time between the end of Stage 1 and the beginning of Stage 3. Stage 3, which is an almost linear change over time, is readily characterized as zero-order kinetics, *i.e.*, the slopes of the straight-line regions can be compared, to yield rate constants. Stage 4 can also be treated in this manner.

The results of this analysis are summarized for waxy-corn starch and potato starch in Table III. They confirm what is apparent from the original data, namely, that all four stages are more rapid for potato starch than for waxy-corn starch.

We now turn to a possible model of the mechanistic events that are responsible for the four stages. At this time, there are insufficient data to say with certainty what is happening at each stage, but it is possible to build a reasonable model consistent with our current knowledge

First, it should be noted that there have been other reports of multistage kinetics for retrogradation processes[23,24], and these have been interpreted in terms

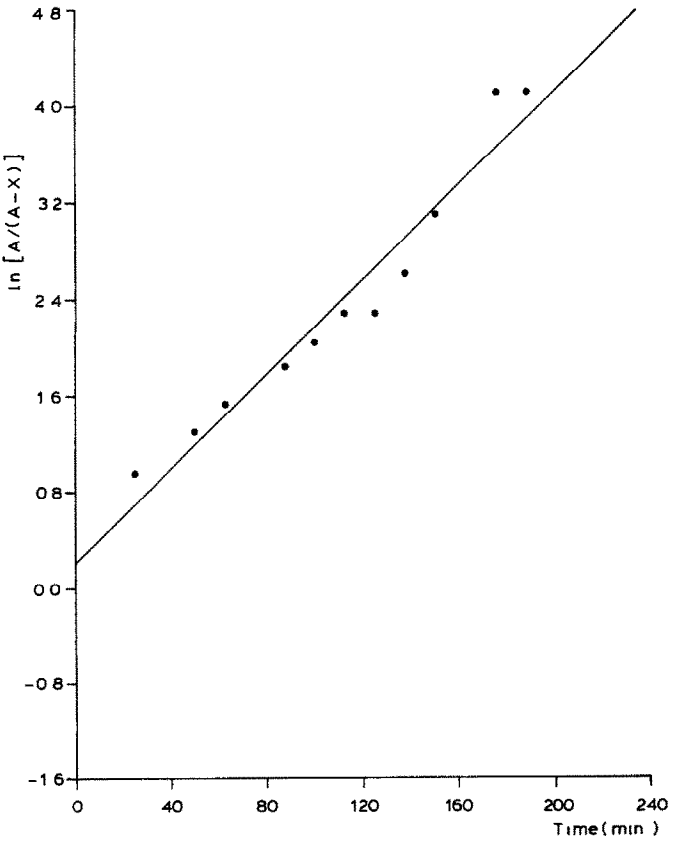

Fig 13. First-order kinetics plot of Stage 1 data for waxy-corn starch in the C–H stretching region of the Raman spectra.

TABLE III

SUMMARY OF KINETIC DATA

Stage	*Parameter*	*Potato starch*		*Waxy-corn starch*	
		C–H	*480 cm^{-1}*	C–H	480 cm^{-1}
1	First-order rate constant (min.$^{-1}$)	0 034	0 42	0 02	0 015
2	Plateau time (min.)	63	60	128	113
3	Zero-order rate[a] constant (cm^{-1} min $^{-1}$)	0 021	5.5×10^{-3}	0.014	2.7×10^{-3}
4	Zero-order rate constant (cm^{-1}.min.$^{-1}$)	—	$1\ 35 \times 10^{-4}$	—	3.9×10^{-5}

[a]Zero-order rates depend on actual parameter being measured, and so C–H and 480-cm^{-1} results cannot be compared.

of nucleation followed by growth[23] and, more recently, as gelation followed by crystallization[24].

X-Ray data from our work indicate that crystals do not begin to develop until Stage 3. Thus, any ordering that occurs in Stage 1 must be short-range. Moreover, the X-ray data show continued development of crystallinity in Stage 4.

We can thus understand Stage 1 as being a purely conformational ordering, possibly involving the formation of double helices in amylopectin branches within a single molecule. These would change both the skeletal and C–H modes, without showing true crystallinity. This could also be expected to be a reasonably rapid process, without any measurable induction time.

Stage 2 is the induction time for onset of crystal growth. This is normal for crystallization phenomena. It has proved very difficult to analyze induction times quantitatively between different systems, because such times are very sensitive to the presence of homogeneous or heterogeneous nuclei. The role proposed for amylose (see later) would nonetheless be consistent with an expected decrease in the induction time for potato *vis-à-vis* waxy-corn starch.

Stage 3 is the primary crystallization step. This has a substantial effect on the vibrational modes, and is confirmed by the appearance of diffraction in the X-ray pattern. This crystal growth is also accelerated in the case of potato starch.

Stage 4, the long, slow change in intensity, is characterized by a bigger change in the intensity of X-ray diffraction than Raman scattering. This means that the essential event involves long-range order. Vibrational spectroscopy is rarely sensitive beyond a few nearest neighbors, whereas X-rays are most sensitive to changes involving several unit cells. Stage 4 is thus understood as a crystalline-phase propagation and perfection step.

The results indicate that the role of amylose may be viewed as providing an acceleration, by a template effect, on amylopectin. Amylose itself retrogrades

rapidly, forming an ordered (on the molecular level) matrix which is not necessarily highly crystalline. These ordered chains may act as seed nuclei for regions of the amylopectin, accelerating all steps in the crystallization process.

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