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Potentiometric and Spectrophotometric Studies of Complexes of Hydrolysis Products of Amylose with Iodine and Potassium Iodide

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The formation of coloured complexes in starch–iodine–iodide solutions is of interest in relation to the structure of starch components and also as to the nature of the molecular linkage and optical absorption in iodine–iodide chains. The establishment of the helical structure of the amylose fraction of starch (Hanes, 1937; Freudenberg, Schaaf, Dumpert & Ploetz, 1939; Bear, 1942; Rundle & Baldwin, 1943; Spark, 1952) and the potentiometric titration of amylose with iodine (Bates, French & Rundle, 1943; Gilbert & Marriott, 1948; Higginbotham, 1949) have led to the idea of a collinear core of iodine and iodide molecules arranged end-to-end inside the amylose helix. Recent advances in the enzymic synthesis of a homologous series of straight-chain polysaccharides consisting of glucose residues in α-1:4 linkage (Cori & Cori, 1939; Whelan & Bailey, 1954) have opened up a possible extension of the potentiometric and other methods of investigation for the study of the changes in the iodine–iodide structure and its relation to the change of colour and optical absorption spectra of the complex (Bailey, Whelan & Peat, 1950).

The potentiometric titration of amylose (Bates *et al.* 1943) and of fractionated preparations of hydrolysed amylose (Dvonch, Yearian & Whistler, 1950) with iodine have shown that the bulk of iodine in the complex is absorbed at constant activity, followed by a slow increase, attributed to surface absorption, with the continual increase in the concentration of free iodine as the titration proceeds. For a straight-chain amylose less than *ca.* 100 glucose units it is not possible to distinguish clearly between surface absorption and complex formation. (The order of chain length stated by Dvonch *et al.* is based on earlier possibly inaccurate spectrophotometric estimates of chain length by Swanson, 1948: cf. Bailey *et al.*, 1950; Bailey, 1952.) The iodine activity required for complex formation is a function of the molecular weight of the amylose, being lower for samples of higher molecular weight. Long helices tend to be filled before short ones can absorb appreciably, the stability of the iodine–

iodide core increasing with length. An explanation of this effect has been discussed by Stein & Rundle (1948) in terms of the polarization of a chain of iodine molecules by the electrostatic field induced along the axis of the helix. Baldwin, Bear & Rundle (1944) have reported a fall in the maximum iodine content of the helices with increasing iodide concentration due to a replacement of iodine molecules with iodide or triiodide ions. They suggest that, the adsorption of the iodine molecules attached to the ends of the helix being weak, the activity as determined by potentiometric measurements increases before the last possible iodine has entered the helix, causing the apparent amount of iodine adsorbed to be less than that determined by spectrophotometric titration. In this latter method all the iodine is assumed to be in complex formation if it contributes to a rise in the optical absorption of the solution over that of an iodine–water solution. In calculating from the spectrophotometric data the amount of iodine adsorbed, the contribution of the free iodine in solution has been neglected. This error is slight in the case of amylose but for shorter straight-chain polysaccharides the increase in free-iodine activity necessary for complex formation increases the contribution appreciably.

Gilbert & Marriott (1948) have studied the adsorption isotherm of amylose in the region of very small percentage adsorption over a range of low iodide concentration (10^{-3}M–10^{-4}M) using a differential potentiometric method. Under these conditions the amylose complex is comprised mostly of groups of the type ($3I_2$–$2I^-$), the number of iodine molecules in the complex increasing with increase in iodine activity. If the iodide concentration is increased the ratio of iodine molecules to iodide ions incorporated tends towards 1, i.e. $(I_3^-)_n$, accompanied by the appearance of a slightly purple tinge to the blue of the complex. Gilbert & Marriott have suggested that forms of starch or degraded starch which form red or purple complexes with iodine are unable to stabilize a long chain of iodine and iodide atoms and that an extension of the method of iodine titration may reveal in such cases complexes of less than eight atoms of iodine or iodide ions per molecule. It is interesting to compare these observations with those of Godina & Faerman (1950) on the crystalline complexes formed only in the presence of iodide ions by an ethanolic solution of iodine with quinine bisulphate. The colour of these compounds varies from red through purple to blue as the I_2:HI ratio changes from 1 to 2. A similar shift of the absorption peak to longer wavelengths with increasing iodine concentration is also observed with polyvinyl alcohol films. As Rundle, Foster & Baldwin (1944) have stated, the general appearance of pleochroism suggests the existence of a parallel array of iodine molecules in all these complexes, amylose solutions being unique by virtue of a single long molecule forming a helical envelope for the iodine molecules, while the other crystalline substances consist of an aggregate of orientated crystals with iodine adsorbed between the molecules (cf. Meyer & Bernfeld, 1941).

Electrophoretic fractionation of α-amylase-hydrolysed amylose in the presence of I_2–KI solution has been carried out on a continuous preparative scale. The degree of polymerization (DP) of the blue-, red-, and orange-staining fractions ranged from 90 to 40, 40 to 25, 25 to 10 respectively (Mould & Synge, 1954). An attempt towards the characterization of their iodine–iodide complexes has been made by means of potentiometric titration with iodine and observations on the absorption spectra. These measurements are of an exploratory character only, but a critical review of the encouraging results and of certain technical difficulties that have arisen seems appropriate in view of the possibility of similar investigations being extended to enzyme-synthesized straight-chain polysaccharides. The red-staining fraction is no more, and probably less, heterogeneous than preparations so far obtained by direct synthesis of a mixture of similar average DP (Whelan & Bailey, 1954).

METHODS

Potentiometric titrations with iodine

Titrations of the blue- and red-staining fractionated polysaccharides were carried out in the same differential potentiometric-titration apparatus, the technique being essentially similar to that described by Gilbert & Marriott (1948). Two solutions of KI, buffered to about pH 6, one containing the polysaccharide, were joined by a salt bridge and iodine solution was added to each in such quantities that the potential between two platinum electrodes immersed in the respective solutions remained less than 0·05 mv.

Fractionated blue-staining material (20·0 mg.) was dispersed in 4 ml. N-NaOH at room temperature, neutralized with 5·2 ml. N-H_3PO_4 and made up to 100 ml. of solution. Of this solution 90 ml. were made up to 1 l. with KI solution to give final concn. 0·001 M-KI in one of the cells (final pH 6). An identical solution (without polysaccharide) was prepared for the titration cell. Both cells were brought to 20°, the electrodes and salt bridge inserted, and 0·00532 M-I_2, 0·01 M-KI added in small increments to both cells from an Agla micrometer syringe (Burroughs Wellcome and Co.). Following the addition of iodine to the cell containing polysaccharide, the titration cell was balanced to within 0·05 mv, the conditions of balance becoming constant after 10 min., or longer, as the iodine activity increased. A similar procedure was followed for the red-staining material, except that 50·0 mg. polysaccharide were used in the preparation of the solutions.

Owing to the high concentration of iodine required before appreciable iodine adsorption takes place it was necessary to add comparatively large amounts of I_2–KI solution to the cells, with a consequent appreciable increase in KI concen-

tration and volume. Corrections have been applied for these cumulative effects on the balance condition in calculating the free-iodine concentration in the polysaccharide cell.

The sensitivity of detection of the null point is very much lowered at high iodine concentration. For accurate work it would therefore be necessary to use an electrometer circuit capable of detecting a potential difference of the order 0·01 mv (cf. Anderson & Greenwood, 1953). In order to minimize the effect of increasing KI concentration with addition of iodine the titration would have to be performed in stages, i.e. starting at an arbitrary point in complex formation with the initial iodide concentration adjusted to 0·001 M. A titration at a lower iodide concentration, e.g. 0·0001 M, would require a complete preliminary titration and then even more careful repetition over very short ranges of iodine activity at the fixed iodide concentration.

The ultraviolet absorption spectrum of amylose–iodine–iodide complex

Amylose (alumina–thymol precipitated, 10 mg.) from potato starch was dispersed on gentle warming in 1 ml. N-NaOH, the solution neutralized by N acetic acid and made up to 50 ml. at pH 6·0. Samples (5 ml. 1 mg. amylose) were diluted to 25 ml., a requisite amount of 0·0032 M-KI being added to make the final solution 0·001 M in KI after the addition of 0·30, 0·25, 0·20 or 0·15 ml. of a solution of 0·2% I_2 in 1% KI to the samples covering a range of iodine concentration from $9{\cdot}46 \times 10^{-5}$ M to $4{\cdot}73 \times 10^{-5}$ M. The flasks were shaken for 7 min., allowing equilibrium conditions of adsorption to be reached, and an absorption curve plotted from 260 to 400 mμ. in a 1 cm. cuvette on a Beckman DU Quartz Spectrometer. The same time was allowed for each plot so that at any particular wavelength each solution had been under the same conditions since the addition of iodine.

The absorption curves of identical solutions under comparable conditions were plotted from 400 to 700 mμ. Absorption curves from 260 to 400 mμ. for iodine solutions covering a range of concentration of iodine from $9{\cdot}46 \times 10^{-5}$ M to $1{\cdot}81 \times 10^{-5}$ M in 10^{-3} N-KI were also plotted.

The whole experimental procedure was repeated using solutions containing 2 mg. amylose in 25 ml. with iodine concentrations ranging from $12{\cdot}6 \times 10^{-5}$ M to $4{\cdot}73 \times 10^{-5}$ M.

RESULTS

Potentiometric titrations

Representative potentiometric titration curves of adsorbed iodine/mg. polysaccharide against the concentration of free total iodine are shown in Fig. 1, with the change in iodide concentration as titration proceeds indicated. An amylose curve (Gilbert & Marriott, 1948) is included for comparison. The increase in iodine activity with decrease in chain length required for maximum adsorption of iodine is immediately obvious. The gradual increase in iodine adsorption shown by both the blue- and red-staining polysaccharides is probably due to surface adsorption of iodine molecules (Dvonch *et al.* 1950). The tendency for both

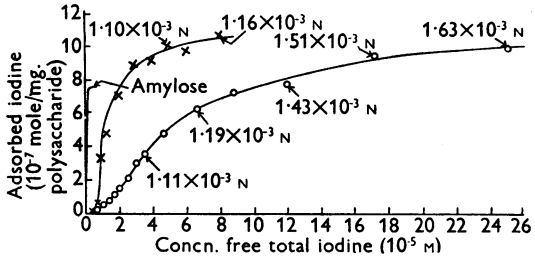

Fig. 1. Titration of fractionated dextrins with iodine in 0·001 M-KI: ×, blue-staining fraction (DP 40–90); ○, red-staining fraction (DP 25–40). The change in iodide concentration is indicated. An amylose titration curve is also shown.

curves to reach about the same maximum height may suggest that maximum surface adsorption may then have been attained. The plot of log (adsorbed iodine) against log (total iodine) is drawn in Fig. 2 for the lower portion of the titration curves where there is less interference from this masking adsorption. The KI concentration can be regarded as constant in this region, and following Gilbert & Marriott's treatment the number of molecules (x) of iodine in the complex is given by the relation

$$x = \frac{\mathrm{d} \log a}{\mathrm{d} \log [I_2]},$$

where a = number of moles of iodine bound in complex/g. polysaccharide. In the case of the blue-staining polysaccharide, it is seen that an initial slope corresponding to $x = 2$ changes at higher concentration to $x = 3$. The characteristic blue-complex coloration is first faintly visible when the iodine activity corresponds to the change in slope, thereafter gradually increasing in intensity. Over this range of activity complex formation involving three molecules of iodine would appear probable. Later, as shown by the non-linearity of the log/log plot, this figure falls off with increasing iodine activity and is presumably affected by surface adsorption. The change $x = 2$ to $x = 3$ is similar to that observed in the early stages of blue-complex formation with amylose (Gilbert & Marriott, 1948). The apparent absence of higher complexes might be expected as the shorter helices are unable to stabilize such a long iodine–iodide chain as amylose, but, considering the heterogenity of the fraction and other factors, the experimental evidence is insufficient to provide a conclusive argument.

For the red-staining polysaccharide, the initial slope corresponds to $x = 1$, changing at higher concentrations to $x = 1{\cdot}8$. Again the appearance and intensification of the colour occurs along the second linear portion of the log/log plot. Titration of another sample, prepared from solution by butanol

precipitation instead of ethanol precipitation (Mould & Synge, 1954), gave a slope of $x = 1{\cdot}15$ changing to $x = 2{\cdot}22$. There is reason to believe (Dvonch *et al.* 1950) that the butanol complex aids helical formation of the chains. The experimental values therefore suggest that the complex formed by the red-staining straight-chain polysaccharides contain two molecules of iodine/molecule polysaccharide. This figure may be compared with $x = 1{\cdot}2$ for amylopectin saturated with iodine (Hybart, 1952) as indicative of a difference in the mechanism of complex formation encountered in branched-chain polysaccharides.

The evaluation of the amount of iodide ion in the complex by varying the iodide concentration has not been attempted. This so far can only be inferred qualitatively from the electrophoretic behaviour of the fractions (Mould & Synge, 1954), since the negative charge and mobility in an electric field of the polysaccharide in iodine–iodide solution presumably originates from the iodide ion content, except in so far as the net charge may be modified by interaction in the collinear chain of I_2 molecules and I^- ions.

Owing to experimental difficulties in controlling the iodide concentration no detailed investigation of the orange-staining complex was possible. The minimum concentrations of iodine required for saturation in 0·001 M-KI are $1{\cdot}0 \times 10^{-5}$ M, $3{\cdot}2 \times 10^{-5}$ M and about $15{\cdot}0 \times 10^{-5}$ M respectively for the blue, red and orange complexes.

The absorption spectra of amylose–iodine–iodide complexes

The change in colour from blue to red or the shift of the absorption band to shorter wavelengths during the hydrolysis of amylose has been shown to bear a relationship to the average chain length of the straight-chain polyglucosides present (Swanson, 1948; Bailey *et al.* 1950), although it would appear that a measure of the wavelength of maximum absorption can only be used as a rough guide to the average molecular weight. The extinction coefficients at the absorption peaks also increase with increasing chain length and higher stability of the iodine complex. With branched-chain polysaccharides of the amylopectin type, if the concentration of iodine is sufficiently high, iodine can be adsorbed to the same extent as on amylose mainly by surface adsorption of single molecules or triiodide ions. Higginbotham (1949) has divided the absorption spectrum of amylopectin and glycogen complexes into two components, one with a peak at 540 mμ. due to a short-chain helical complex and the other with a peak of the order of 400 mμ. assumed to be due to adsorbed triiodide ion.

From a review of the literature it would appear that little attention has been given to the near-visible (200–400 mμ.) ultraviolet absorption spectra of the polysaccharide–iodine complexes. The accuracy of measurement in this region of the spectrum is hampered by the large correction which

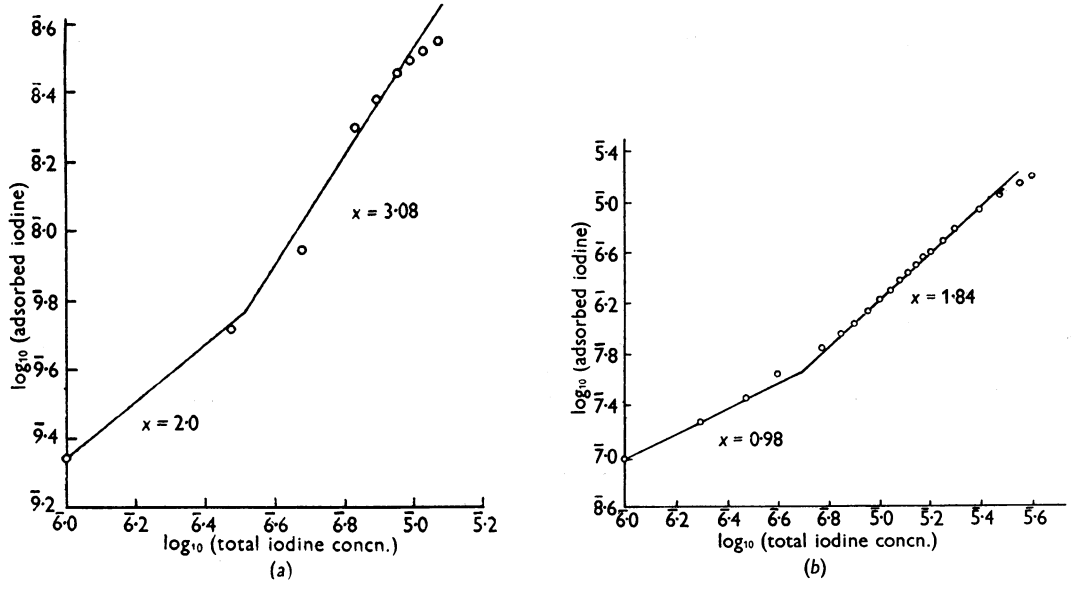

Fig. 2. Plot of $\log_{10}$ (adsorbed iodine) against $\log_{10}$ (concentration of total iodine). Units and conditions as in Fig. 1. (*a*) Blue-staining fraction; (*b*) red-staining fraction.

must be made for the iodine blank and the wide range of concentrations of iodine, iodide, and polysaccharide used by various workers has led to confusion. Iodine in aqueous iodide solution exhibits three well-defined absorption bands at 226, 287 and 353 mμ., the last two being attributed to the triiodide ion (Awtrey & Connick, 1951). The heights of these bands are considerably modified by the presence of polysaccharide (Lampitt, Fuller & Goldenberg, 1941) and Bebbington, Bourne & Wilkinson (1952) have reported the complete suppression of the 287 mμ. band with a slight enhancing of the 353 mμ. band by amylose (see Cramer, 1951). If the amount of free iodine and iodide in the solution is known there is little difficulty in estimating the contribution of the unadsorbed iodine to the absorption spectrum and this can then be subtracted from the observed total spectra to determine the contribution of the absorption spectra of the iodine–polysaccharide complex. A comparison with the absorption spectra of free iodine in solution is likely to be of interest in view of the various possibilities as to the nature of bonding of iodine in the complex.

If the iodine concentration is sufficient to saturate the amylose, the absorption peak in the visible (640–660 mμ.) should remain of constant height. A slight falling off was observed at the lowest concentration of $4{\cdot}73 \times 10^{-5}$ M-I_2. Allowing for a concentration of $0{\cdot}2 \times 10^{-5}$ M-I_2 (Gilbert & Marriott, 1948) to be necessary for saturation, it may roughly be inferred that the amount of iodine adsorbed was less than $12{\cdot}0 \times 10^{-7}$ moles I_2/mg. polysaccharide. In the ultraviolet there was a gradual fall in the levels of the absorption peaks, especially at the lowest concentration of iodine when the 287 mμ. peak was lower than the 353 mμ. peak. This is in agreement with Bebbington *et al.* (1952).

Assuming arbitrary values for the amount of molecular iodine taken up by the amylose from $6{\cdot}0 \times 10^{-7}$ moles/mg. to $12{\cdot}0 \times 10^{-7}$ moles/mg., the residual concentrations of free iodine in 0·001 M-KI were calculated. The appropriate absorption values for wavelengths 287 and 353 mμ. were intrapolated from the calibration curves for iodine solutions and subtracted from the observed value, at the same wavelength, of the amylose–iodine complex absorption curves. Since the polysaccharide was apparently saturated at all the iodine concentrations the difference at any wavelength should be constant when the correct iodine adsorption has been applied. The best fit lies about $7{\cdot}7$–$8{\cdot}0 \times 10^{-7}$ moles/mg. and the value 8×10^{-7} moles/mg. has been assumed with regard to the experimental errors involved. Complete light-absorption curves for the residual concentration of free iodine were then calculated based on this value for the iodine adsorption and subtracted from the observed curve in the presence of polysaccharide to determine the actual contribution from the amylose–iodine–iodide complex itself. It will be seen that reasonably good agreement is obtained over the range of iodine concentration used (Fig. 3).

Very similar conclusions were reached at the higher amylose concentration. From the visible absorption curves there was little doubt that saturation conditions were not reached below $7{\cdot}88 \times 10^{-5}$ M-I_2, i.e. the iodine adsorption lay between $10{\cdot}0 \times 10^{-7}$ and $8{\cdot}0 \times 10^{-7}$ moles/mg. The best statistical fit was obtained for an iodine adsorption of 9×10^{-7} moles/mg. and the contribution of the amylose–iodine–iodide complex was calculated as before using this value.

In these absorption curves (Fig. 3) there is a very slight change in gradient only in the region 287 mμ. but a pronounced peak at 350 mμ. followed by a minimum at 400 mμ. before rising to the peak in the visible. The extinction is proportional to the concentration of the amylose, which leaves little doubt as to the complete formation of the complex with iodine. The appreciably increased absorption for wavelengths shorter than 300 mμ. due to light scattering and a small superimposed peak at 250 mμ.

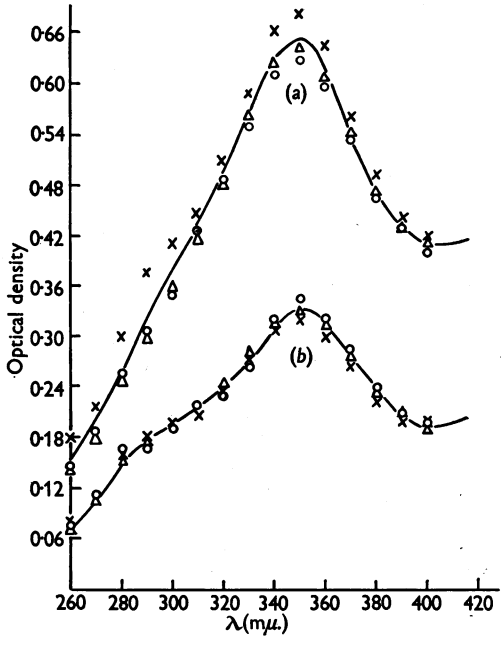

Fig. 3. Ultraviolet absorption curve of amylose–iodine–iodide complex in 0·001 M-KI. (*a*) Amylose concentration, 2 mg./25 ml.: ○, $12{\cdot}60 \times 10^{-5}$ M-I_2; △, $9{\cdot}46 \times 10^{-5}$ M-I_2; ×, $7{\cdot}88 \times 10^{-5}$ M-I_2 (iodine adsorption = $9{\cdot}0 \times 10^{-7}$ moles/mg.). (*b*) Amylose concentration, 1 mg./25 ml.; ○, $9{\cdot}46 \times 10^{-5}$ M-I_2; △, $6{\cdot}30 \times 10^{-5}$ M-I_2; ×, $4{\cdot}73 \times 10^{-5}$ M-I_2 (iodine adsorption = $8{\cdot}0 \times 10^{-7}$ moles/mg.).

(Laurent & Wertheim, 1952) have been neglected but would have the general effect of lowering the apparent absorption on the short-wavelength side of the absorption peak. The amounts of iodine adsorbed are higher than the value $7{\cdot}3 \times 10^{-7}$ moles/mg. quoted in the literature (Gilbert & Marriott, 1948; Bates *et al.* 1943) but can be due to the higher values obtained by the spectrophotometric method as already discussed above (Baldwin *et al.* 1944). This is in agreement with the higher value obtained at a higher concentration of amylose.

DISCUSSION

When iodine molecules are linked by simple covalent bonding (I–I = 2·67 Å) in the gaseous state and also in non-polar solvents (CS_2, CCl_4), a well-defined absorption band occurs at 520 mμ. (Liebhafsky, 1939). In the presence of electron-donor substances I–I is polarized to I^+–I^- or I^-–I^+ (Fairbrother, 1947, 1948). The contribution of these forms to the actual structure present determines the nature of the iodine spectrum. The potential energy above the ground state of the *p*, *s*, valency electrons, which influences light absorption, is increased in the polarized form and shifts the absorption maximum to shorter wavelengths, e.g. 460 mμ. in water, 462 mμ. in ethyl iodide, 490 mμ. in *n*-butyl chloride. Schlamowitz (1951) explains the movement of maximum absorption in amylose–iodine complexes towards the red with increasing chain length in terms of the gradually increasing effect of the supposed non-polar environment of the inside of the amylose helix on the adsorbed iodine molecules, but it is difficult to see how absorption maxima at wavelengths greater than the limiting value of 520 mμ. set by the unpolarized molecule are obtained. He concludes that the absorption spectrum of a polysaccharide–iodine complex is essentially a spectrum of the free iodine molecule.

In the case of short polyglucoside chains or branched polysaccharides of the amylopectin or glycogen type where the iodine bonding is weak and the hydrophobic region of the core very small, the absorption spectra of the polysaccharide complex would be expected to tend towards those exhibited by iodine or triiodide ion in aqueous solution. Essential consideration must be given to the presence of I^- or I_3^- in the adsorbed complex as indicated by the potentiometric titration (Gilbert & Marriott, 1948) and the mobility of the complex in an electric field (Mould & Synge, 1954). A linear configuration for I_3^- as a trigonal pyramid with the two external iodine atoms at the two pyramidal apices and three unbonded electron pairs in the equatorial plane around the central iodine atom has been established (Wyckoff, 1920; Mooney, 1935, 1937). The interaction of the I^- ion with a highly polarizable I_2 molecule leads to a small contribution of covalent bonding between the I^- and the nearer iodine of the I_2 group, giving the resonating structure

$$\overbrace{I^- \cdots \overbrace{I\text{–}I}^{2{\cdot}82\text{ Å}}}^{3{\cdot}10\text{ Å}} \qquad \overbrace{\overbrace{I\text{–}I}^{2{\cdot}82\text{ Å}} \cdots I^-}^{3{\cdot}10\text{ Å}}$$

Two absorption bands always appear, e.g. 287, 353 mμ., whenever this configuration is found (Allsopp, 1937; Potterill & Walker, 1937).

Stein & Rundle (1948) have accounted for the stability of the complex increasing with length of helix, in terms of the action of the dipole field of the helical polymer acting along the axis on a highly polarized array of halogen molecules. The field tends to reduce the van der Waals distance between neighbouring iodine molecules, and resonance occurs between the bonded and non-bonded iodine atoms so that the modified van der Waals and bonded distances tend to approach each other. A periodicity along the chain of 6·2 Å (2 × 3·1 Å) as found by West (1947) for other polymer–iodine complexes is not impossible according to this theory. Their calculations are, however, only strictly applicable for the case of adsorption of iodine vapour by solid dry amylose. Hach & Rundle (1951), following on studies of the I_5^- structure, have postulated that the distances apart of the iodine and iodide atoms in a linear resonating array can be between 3·0 Å and 3·1 Å. Cramer (1951) has reported a periodic distance of 3·06 Å in precipitated iodine–iodide complexes of Schardinger dextrins.

It appears likely that the suppression of the 287 mμ. peak in the amylose–iodine–iodide complex may be the result of change in electron orbital configuration due to the modified bond distance and certainly shows the absence of individual I_3^- structure. The resonance-energy contribution lowers the energy levels of the valency electrons and may be sufficient to shift the absorption maximum towards the longer wavelengths in the visible spectrum. It is suggested that with decreasing length of the helix the stable resonating extended chain breaks up into discrete complex groups and gradually increasing polarization of the I_2 molecules by the I^- ions now assumes a predominant role. This would have the effect of shifting the absorption maxima to shorter wavelengths and it would also be expected that the 287 mμ. peak would again show up as the I_3^- type structure gradually reappeared.

A spectrophotometric investigation of the red-staining polysaccharide (DP 34) similar to that carried out with amylose was inconclusive and an accurate absorption curve for the complex was not obtained. This was due to extensive surface-adsorbed or loosely bound iodine at the high free-

iodine concentration, also evident in the potentiometric titrations. It does not contribute to the absorption band in the visible but does contribute to the bands in the ultraviolet. The much smaller amount of iodine taken up in complex formation gave a small contribution to the visible absorption with a peak at about 510–520 mμ. The agreement of this value with that arising from covalent-bonded iodine is fortuitous as with still shorter chain lengths this value progressively falls to 490 mμ. (Whelan & Bailey, 1954). From arbitrary assumptions as to the amount of iodine adsorbed in the true helical complex taken from the potentiometric curves it would appear that the complex has a pronounced double peak in the ultraviolet (Fig. 4). This was borne out by changes in the maxima of the peaks when the polysaccharide was added in increasing amounts to iodine solution of constant concentration. The height of the 353 mμ. peak first fell and then rose with increased polysaccharide concentration. Observation of the corresponding changes in the 287 mμ. peak was not made, suitable light-absorption cells of small cross-section not being available. The effect may readily be explained by the induced formation of I_3^- type structures within the short helices of the polysaccharide increasing the proportion of the I_3^- ion in the polysaccharide–iodine–iodide solution more than would be expected under normal dissociation-equilibrium conditions. The field of the helix is presumably not long enough to stabilize a long chain and the interaction of the complex ions with each other are not enough to modify the I_3^- structure to any extent.

Cramer & Herbst (1952) have recently considered the formation of the absorption maxima exhibited by polysaccharide–iodine complexes in terms of the behaviour of a resonating iodine chain acting as a 'one-dimensional metal' (Kuhn, 1949). The calculated absorption maxima are of the right order of wavelength but decrease too rapidly with decrease in length of the iodine–iodide chain. This also may indicate that the short complexes cannot be considered as stable extended resonating chains.

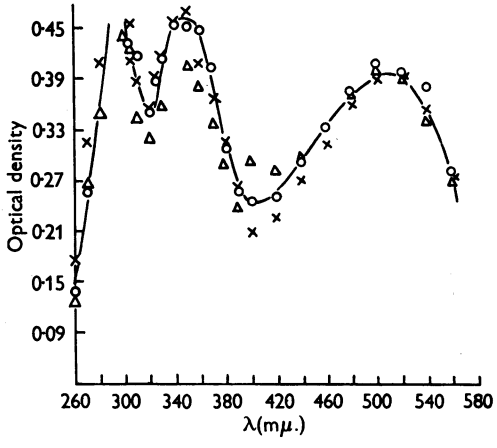

Fig. 4. Absorption curve of red-staining dextrin, DP 25–40, in 0·001 M-KI. Concentration of polysaccharide, 1 mg./25 ml.: ○, $25{\cdot}20 \times 10^{-5}$ M-I_2; △, $22{\cdot}08 \times 10^{-5}$ M-I_2; ×, $18{\cdot}92 \times 10^{-5}$ M-I_2. Iodine adsorption estimated from Fig. 1.

SUMMARY

1. The potentiometric titration with iodine of electrophoretically fractionated dextrins from α-amylase-hydrolysed amylose is described. The complex $3I_2$–yI^- is formed by the blue-staining fraction, degree of polymerization (DP) 40–90, and the complex $2I_2$–$y'I^-$ by the red-staining fraction, DP 25–40.

2. Experimental difficulties due to the high free-iodine concentration required for complex formation are discussed.

3. The ultraviolet absorption spectrum of the amylose–iodine–iodide complex has been determined and changes in the absorption spectrum given by the complexes formed by dextrins of shorter chain length are discussed.

4. It is suggested that with amylose the iodine–iodide chains form a stable resonating extended chain, but with decrease in length of helix this breaks up into discrete complex groups and the gradually increasing polarization of the I_2 molecules by I^- ions has the effect of shifting the absorption maxima to shorter wavelengths. From spectrophotometric data the presence of an I_3^- structure in the red-staining complexes is possible.

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Route of Absorption and Distribution of Oleic Acid and Triolein in the Rat

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During the last few years a better understanding of the process of intestinal fat absorption has been gained, largely with the aid of isotopically labelled material, in combination with the technique of Bollman, Cain & Grindlay (1948) for collecting intestinal or thoracic lymph from unanaesthetized rats. The results of these investigations have shown that after absorption long-chain saturated fatty acids are mainly transported via the intestinal lymph as triglycerides both in rats and cats (Bloom, Chaikoff, Reinhardt, Entenman & Dauben, 1950; Bergström, Borgström, Carlsten & Rottenberg, 1950; Bloom, Chaikoff, Reinhardt & Dauben, 1951; Chaikoff, Bloom, Stevens, Reinhardt & Dauben, 1951; Bloom, Chaikoff & Reinhardt, 1951; Borgström, 1951, 1952*a*, *b*, *c*; Bergström, Borgström & Carlsten, 1954). Fatty acids with 10 or less carbon atoms are, however, mainly transported from the intestine via the portal blood vessels (Bloom, Chaikoff & Reinhardt, 1951; Kiyasu, Bloom & Chaikoff, 1952) largely in free form (Borgström, to be published). For summaries see Bergström & Borgström (1953, 1954).

The higher saturated fatty acids are thus not absorbed via different routes according to the state in which they enter the intestinal mucosa, i.e. as free acids or esterified with glycerol, as postulated in the original fat–fatty acid partition theory of Frazer (Frazer, 1938, 1943, 1946, 1948*a*, *b*, 1950, 1951, 1952).

There is also much experimental evidence that the unsaturated long-chain fatty acids are mainly absorbed via the lymphatics irrespective of whether they are fed as free acids or glycerides.

Munk (1880) fed oleic acid to dogs with a cannulated thoracic duct and found that there was a considerable increase in the neutral fat of the lymph. Munk & Rosenstein (1891) fed olive oil, oleic and erucic acid to a patient having a lymphatic fistula and obtained a high recovery of the ingested fat in the lymph. Freeman & Ivy (1935) obtained the same results after feeding olive oil to dogs. Bollman, Flock, Cain & Grindlay (1950) observed a marked increase of glycerides in the lymph after feeding oleic acid to dogs. Reiser & Bryson (1951) and Reiser, Bryson, Carr & Kuiken (1952) fed linoleic acid with conjugated double bonds to rats having a thoracic duct fistula and recovered part of the labelled acid in the lymph fat both after free acid and glyceride ingestion.