232. α-1,4-*Glucosans. Part XI.*[1] *The Absorption Spectra of Glycogen- and Amylopectin–Iodine Complexes.*

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The absorption spectra of the iodine complexes of a large number of starch-type polysaccharides have been measured. Amylopectins and glycogens show maximum absorption at *ca.* 540 and *ca.* 460 mμ respectively. This dissimilarity may be correlated with differences in type of iodine-binding arising from variations in the average length of the interior chains of the two polysaccharide-types. Mammalian glycogens are more iodophilic than invertebrate glycogens; this fact cannot at present be related to known structural features.

Addition of ammonium sulphate and other salts to a polysaccharide–iodine solution causes a marked increase in the iodine-staining power of glycogen, but only a small increase with amylopectin. Under these conditions, the position of maximum absorption is approximately related to the degree of branching in the polysaccharide.

The action of branching or debranching enzymes, which catalyse the synthesis or hydrolysis of α-1,6-glucosidic inter-chain linkages in starch-type polysaccharides may be followed by measurement of changes in the iodine-staining power of the substrate. For example, the activity of liver branching enzyme is determined from the decrease in optical density at 570 mμ of an amylopectin–iodine solution,[2] whilst R-enzyme is assayed from the increase in " blue value " of amylopectin β-dextrin.[3] There is some evidence [4,5] that the iodine-staining power of amylose is related to the average chain length ($\overline{CL}$) or degree of polymerisation ($\overline{DP}$) provided that the amylose molecules do not exceed a certain critical size, although there is no apparent agreement on the actual value of this. However, information on the possible relation between the absorption spectrum of a glycogen- or amylopectin–iodine complex and the degree of branching in the polysaccharide is not available, and we now report such an investigation. A preliminary account of part of this work has been given.[6]

In previous papers of this Series,[1,7,8] the $\overline{CL}$ and β-amylolysis limit of a large number of glycogens and amylopectins, have been reported; from these data, the relative lengths of the exterior and the interior chains have been calculated. The absorption spectra of the iodine complexes of these polysaccharides have now been determined, in conditions similar to those used by Peat and his co-workers [9] in their iodine-staining studies of the action of R-enzyme on glycogen, *i.e.*, the light absorption in the range 400—700 mμ of a solution containing 0·01% of polysaccharide and 0·02% of iodine in 0·2% aqueous potassium iodide was measured on a Unicam S.P. 500 or S.P. 600 spectrophotometer against an iodine–iodide reference solution. Typical curves are shown in Fig. 1; effects due to light absorption by the polysaccharide alone are negligible under these conditions. With glycogens, a wide absorption peak covering 20—30 mμ was frequently obtained, and the $\lambda_{max.}$ quoted represent the mid-points; with amylopectins, a sharper peak was observed and the $\lambda_{max.}$

[1] Part X, Lawrie, Manners, and Wright, *Biochem. J.*, 1959, **73**, 485.
[2] Larner in " Methods in Enzymology," Academic Press Inc., New York, 1955, Vol. I, p. 222.
[3] Hobson, Whelan, and Peat, *J.*, 1951, 1451.
[4] Baldwin, Bear, and Rundle, *J. Amer. Chem. Soc.*, 1944, **66**, 111.
[5] Swanson, *J. Biol. Chem.*, 1948, **172**, 825; Bailey, Whelan, and Peat, *J.*, 1950, 3692; Kerr, Cleveland, and Katzbeck, *J. Amer. Chem. Soc.*, 1951, **73**, 3916.
[6] Archibald, Manners, and Wright, *Biochem. J.*, 1960, **75**, 10P; Liddle, Ph.D. Thesis, Edinburgh 1956; Archibald, Ph.D. Thesis, Edinburgh 1958.
[7] Fleming, Hirst, and Manners, *J.*, 1956, 2831.
[8] Manners and Khin Maung, *J.*, 1955, 867; Manners and Archibald, *J.*, 1957, 2205; Liddle and Manners, *J.*, 1957, 3432.
[9] Peat, Whelan, Hobson, and Thomas, *J.*, 1954, 4440.

values are significant to ± 5 mμ. From the above curves $\lambda_{max.}$ and $E_{max.}$ (the extinction or absorption value at this wavelength) were noted for each polysaccharide, and the results are recorded in Table 1. Control experiments showed that the $\lambda_{max.}$ values were unaffected by variation in the polysaccharide : iodine ratio; the figures as quoted for $E_{max.}$ values are considered to be significant, although slight variation was observed (*e.g.*, in triplicate solutions Floridean starch II had $E_{max.}$ 0·68, 0·67, and 0·72 at 530 mμ).

The general results indicate a marked difference in the absorption spectra of the iodine complexes of amylopectins and glycogens. The former complexes show much stronger absorption ($\lambda_{max.}$ 530—550 mμ, $E_{max.}$ 0·8—1·2) than those of glycogen ($\lambda_{max.}$ 420—490 mμ, $E_{max.}$ 0·1—0·4) even though the $\overline{CL}$ values of some amylopectins (*ca.* 20) are not greatly different from those of certain glycogens. This is especially true of malt amylopectin and horse muscle glycogen (Fig. 1).

The iodine absorption spectra of individual glycogens vary considerably and, for aqueous solutions, they appear to depend upon the biological source and not on the average

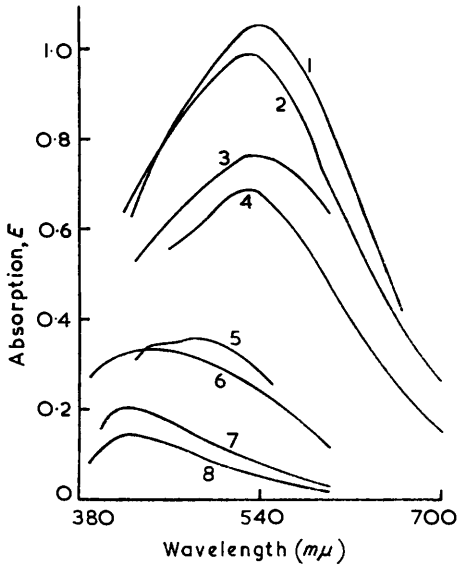


FIG. 1. *Light-absorption curves of iodine-stained polysaccharides.*

1, Waxy sorghum starch; 2, waxy maize starch; 3, malt amylopectin; 4, Floridean starch; 5, horse muscle glycogen; 6, rabbit liver glycogen; 7, skate liver glycogen; 8, *Mytilis edulis* glycogen.

or exterior chain length. There is no correlation between the $\lambda_{max.}$, $E_{max.}$, or extinction at a particular wavelength (*e.g.*, 460 mμ) and the branching characteristics. It follows that increases in the absorption spectra of the iodine complexes of liver glycogen isolated from animals in different metabolic conditions [10] are not necessarily due to changes in molecular structure. Previous observations [11] that fish and invertebrate glycogens give yellow-brown stains with iodine whilst mammalian samples give red-brown colours have been confirmed. In general, the iodine-staining power increased in the order, glycogen β-limit dextrin, invertebrate glycogen, mammalian liver glycogen, and mammalian muscle glycogen. This variation of iodine-staining power with biological source is an important factor in iodine-staining methods for the determination of glycogen, and separate calibration curves are suggested for glycogen samples from different species.[12]

The iodine-staining of the Floridean starches and sweet-corn polysaccharides is of special interest. The algal polysaccharides resemble typical glycogens rather than amylopectins with regard to degree of branching,[7] and yet give appreciably more intense iodine-stains than do the animal polysaccharides. A sample of Floridean starch examined by

[10] Chapman, Fells, and Chaikoff, *Experientia*, 1955, **11**, 283.
[11] Bell and Kosterlitz, *Biochem. J.*, 1935, **29**, 2027; Bell, *ibid.*, 1935, **30**, 2144.
[12] Van der Vies, *Biochem. J.*, 1954, **57**, 410.

TABLE 1. *Iodine-staining properties of polysaccharides.*

Polysaccharide	$\lambda_{max.}$ (mμ)	$E_{max.}$	$\overline{CL}$ *	$\overline{ECL}$ †	$\overline{ICL}$ †
Glycogens:					
Arenicola	420	0·2	11[e]	7—8	2—3
Ascaris lumbricoides	435	0·2	12[a]	8	3
Cardium	420	0·1	8[e]	3—4	3—4
Cat liver IV	465	0·3	13[e]	9—10	2—3
Cock liver	440	0·1	13[e]	7—8	4—5
Fœtal pig liver	440	0·5	11[e]	8	2
Helix pomatia II	425	0·1	7[a]	4	2
Horse muscle	460	0·3	11[a]	7	3
Human liver I	460	0·2	14[b]	9	4
II	430	0·05	6[b]	2	3
Mytilus edulis I	435	0·1	12[a]	7—8	3—4
II	440	0·2	16[a]	10—11	4—5
IV	450	0·1	12[e]	8—9	2—3
VII	420	0·2	13[e]	8—9	3—4
VIII	420	0·2	13[e]	8—9	3—4
IX	430	0·2	10[e]	7—8	1—2
X	420	0·2	14[e]	8—9	4—5
Oyster	440	0·2	10[e]	6—7	2—3
Rabbit liver I	455	0·2	13[e]	5—6	6—7
IV	460	0·3	13[e]	8—9	3—4
V	450	0·3	14[e]	9—10	3—4
VIII	460	0·2	13[e]	9	3
IX	485	0·4	13[e]	9	3
X	475	0·3	12[e]	8—9	2—3
Rabbit muscle I	490	0·4	12[a]	8	3
III	490	0·3	13[c]	8—9	3—4
Skate liver	420	0·2	13[e]	8—9	3—4
Trichomonas gallinae II	440	0·3	13[8]	8—9	3—4
Yeast (brewer's)	430	0·3	13[8]	8	4
Amylopectins:					
Potato I (King Edward)	540	1·3	23[e]	14—15	7—8
II (Great Scot)	555	1·2	24[d]	17	6
Protozoal	530	1·3	22[6]	15—16	5—6
Waxy maize starch I	530	1·0	22[6]	14—15	6—7
II	530	0·9	21[6]	15	5
Waxy sorghum starch II	535	1·0	22	15	6
Other polysaccharides:					
Ascaris glycogen β-dextrin	430	0·1	6—7	2—3	3
Fœtal sheep liver glycogen β-dextrin	430	0·1	6—7	2—3	3
Floridean starch I	500	0·8	9[7]	6—7	1—2
II	530	0·7	12[7]	7	4
III	530	0·6	13[7]	7—8	4—5
Helix glycogen β-dextrin	430	0·03	4—5	1—2	2
Rabbit liver glycogen dextrin	460	0·3	9	5	3
Waxy maize starch β-dextrin	530	0·9	10	2—3	6—7
Waxy sorghum starch β-dextrin	540	0·8	12	2—3	8—9
Zea mays *polysaccharides*:					
Phytoglycogen A	450	0·2	13[14]	9	3
B	430	0·2	7[14]	5	1
Fraction 55—60	480	0·2	10[14]	—	—
60—65	450	0·2	11[14]	—	—
65—70	445	0·1	10[14]	—	—

* Average chain length, determined by periodate oxidation (superscript numbers refer to the previous results and the superscript letters to the following references: *a*, Bell and Manners, *J.*, 1952, 3641; *b*, Calderbank, Kent, Lorber, Manners, and Wright, *Biochem. J.*, 1960, **74**, 223; *c*, Manners and Wright, unpublished work; *d*, Fleming and Mercer, unpublished work; *e*, Liddle and Manners, *J.*, 1957, 3432).

† $\overline{ECL}$, exterior chain length, *i.e.*, no. of glucose residues removed by β-amylase + 2·5; $\overline{ICL}$, interior chain length, *i.e.*, $\overline{CL} - \overline{ECL} - 1$.

Peat, Turvey, and Evans [13] which had $\overline{CL}$ 15 also showed $\lambda_{max.}$ 530 mμ. The water-soluble polysaccharides from *Zea mays* are structurally indistinguishable from animal glycogens,[14] and the data in Table 1 are in accord with this finding.

In contrast to the glycogens, amylopectins from various plant and protozoal starches showed similar $\lambda_{max.}$ values; there was some variation in the extinction at 680 mμ and this is attributed to the presence of small amounts of amylose as impurity in the amylopectin samples. For example, although waxy maize starch is normally considered to be free from amylose, potentiometric titration [15] has indicated the presence of 1·4% of linear polysaccharide in sample I. However, control experiments have shown that the presence of even 5% of amylose impurity does not appreciably affect the position of $\lambda_{max.}$.

The iodine-staining properties of glycogen and amylopectin show a more marked difference after β-amylolysis. With glycogen, both $\lambda_{max.}$ and $E_{max.}$ are decreased, whereas amylopectin β-dextrin has the same $\lambda_{max.}$ as the original polysaccharides. It is clear

FIG. 2. *Relation between interior chain length of a polysaccharide and* $\lambda_{max.}$ *of the iodine complex.* ● *and* ○ *represent glycogen and amylopectin respectively.*

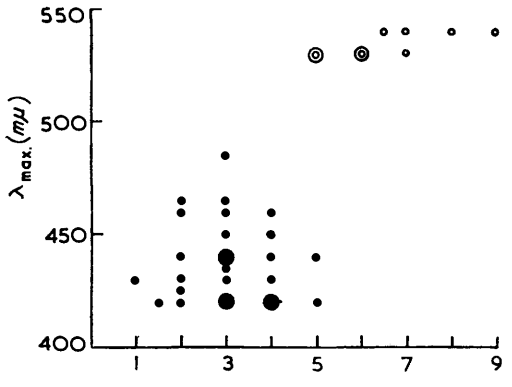
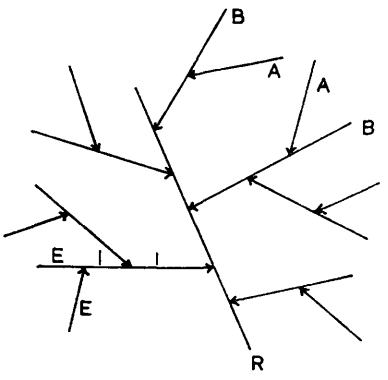


FIG. 3. *Multiply-branched structure for amylopectin and glycogen.*



R, *Reducing-group*; E, *exterior chain*; I, *interior chain*; A, A-*chain*; B, B-*chain*.

that in amylopectin, the $\lambda_{max.}$ of the iodine complex is not related to the length of the exterior chains. In contrast, there is evidence [15] that the iodine-binding power of branched α-1,4-glucosans, as determined by potentiometric titration, increases with the length of the exterior chains.

TABLE 2. *Calculated * lengths of A- and B-chains.*

	$\overline{CL}$	β-Amylolysis limit (%)	Average lengths A-chain	B-chain
Amylopectin	20 †	57 †	14	26
Glycogen	12 †	45 †	8	16

* The exterior A- and B-chain stubs in a β-dextrin are assumed to contain 2—3 glucose residues.
† Typical experimental results.

The above results indicate that the nature of the iodine-binding in glycogen and in amylopectin is different. Higginbotham [16] has suggested that amylopectin binds iodine partly by the adsorption of iodine molecules or tri-iodide ions, and partly by a mechanism in which iodine molecules are arranged endwise and axially inside a series of helices of

[13] Peat, Turvey, and Evans, *J.*, 1959, 3341.
[14] Peat, Whelan, and Turvey, *J.*, 1956, 2317.
[15] Anderson and Greenwood, *J.*, 1955, 3016.
[16] Higginbotham, *Shirley Inst. Mem.*, 1949, **23**, 171.

α-1,4-linked glucose residues. Each coil of the helix is believed to contain six glucose residues and one iodine molecule.[4] The smallest amylose-type molecule which gives a colour with iodine probably contains *ca.* 18 glucose residues,[17] *i.e.*, a sequence of three helical coils is required.

One possible explanation for the difference in the iodine-staining properties of amylopectin and glycogen is the characteristic difference in the average length of the interior chains (see Fig. 2) and hence of the B-chains.* By definition, the molecules contain an equal number of exterior and interior chains, and if we assume that amylopectin and glycogen comprise an equal number of A- and B-chains,[18] each A-chain * represents an exterior chain, whilst every B-chain contains one exterior and two interior sections (see Fig. 3). Calculation (Table 2) shows that with amylopectin a considerable proportion of the B-chains would be of a suitable length to assume a helical configuration. In contrast, only a small proportion of the B-chains in glycogen would exceed $\overline{CL}$ 18. It has been suggested that the presence of branch points would interfere with complex formation;[4] however, inspection of models[19] of

TABLE 3. *Iodine-staining properties of glycogens determined by Schlamowitz's method.*[22]

Glycogen	$\lambda_{max.}$ (mμ)	$E_{max.}$	$\overline{CL}$ *	$\overline{ECL}$ *	$\overline{ICL}$ *
Mytilus edulis IX	500	0·5	10 [e]	7—8	1—2
Rabbit muscle I	520	0·3	12 [a]	8	3
Rabbit liver X	500	0·6	12 [f]	8—9	2—3
Tetrahymena pyriformis II	505	0·5	14 [f]	9	4
Trichomonas foetus	505	0·5	15 [f]	11—12	2—3
Trichomonas gallinae II	495	0·6	13 [f]	8—9	3—4
Zea mays					
Phytoglycogen A	550	0·5	13 [14]	—	—
B	530	0·2	7 [14]	—	—

* See Footnotes to Table 1. [f] Manners and Archibald, *J.*, 1957, 2205.

glucose residues in a helix shows that the primary 6-alcohol groups are situated on the outer surfaces of the helix so that the attachment of side-chains to a B-chain should not necessarily affect the interior of the helix.

An additional factor concerns the ability of iodine molecules (size approx 8 Å) and the even larger tri-iodide ion to penetrate the interior of the compact glycogen molecule, as compared with the more open interior of an amylopectin molecule. This is illustrated by the fact that amylopectin β-dextrin, which has the same degree of branching as a normal glycogen, still retains the characteristic amylopectin-type absorption with $\lambda_{max.}$ *ca.* 535 mμ.

The spectral differences between the iodine complexes may, therefore, be related to differences in the average distance between branch points in the interior of the molecules. Since it is now known that glycogen and amylopectin have similar degrees of multiple branching (*i.e.*, the ratios of A-chains to B-chains are similar),[18] this property is not a controlling factor (cf. ref. 15).

It is concluded that, under the above experimental conditions, the absorption spectra of polysaccharide–iodine complexes cannot be directly related to the proportion of α-1,6-glucosidic inter-chain linkages. The use of iodine-staining methods for the study of branching or debranching enzymes cannot therefore give quantitative information on changes in the proportion and distribution of 1,6-linkages in the substrate. Nevertheless, qualitative information can be conveniently obtained. In addition, determination of the $\lambda_{max.}$ of a polysaccharide–iodine complex, together with examination of other properties,

* An A-chain (side-chain) is linked to the molecule only by the reducing group, whilst B-chains (main-chains) which are similarly linked, also have other chains attached to them.

[17] Thoma and French, *J. Amer. Chem. Soc.*, 1960, **82**, 4144.
[18] Peat, Whelan, and Thomas, *J.*, 1956, 3025; Liddle and Manners, *J.*, 1957, 4708.
[19] Cf. Greenwood and Rossotti, *J. Polymer Sci.*, 1958, **27**, 481.

FIG. 4. *Effect of ammonium sulphate on the iodine complex of* Trichomonas gallinae *glycogen. Curves* 1 *and* 2 *were measured for half-saturated and quarter-saturated ammonium sulphate solution. Curve* 3 *shows the spectra in water.*

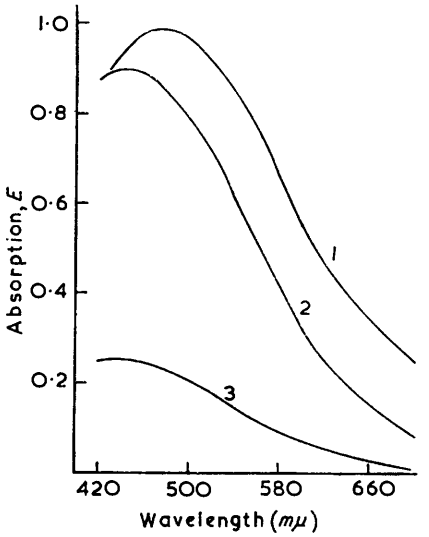


FIG. 5. *Relation between the average chain length of an amylopectin-glycogen type polysaccharide and* $\lambda_{max.}$ *of the iodine complex in half-saturated ammonium sulphate solution.*

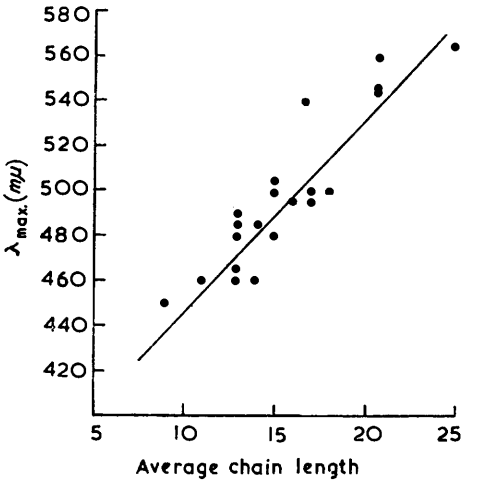


TABLE 4. *Effect of half-saturated ammonium sulphate on the absorption spectra of polysaccharide–iodine complexes.*

Polysaccharide	Water $\lambda_{max.}$ (mμ)	Water $E_{max.}$	NH_4 sulphate $\lambda_{max.}$ (mμ)	NH_4 sulphate $E_{max.}$	$\overline{CL}$ *	$\overline{ECL}$ *	$\overline{ICL}$ *
Glycogens:							
Fœtal sheep liver	450	0·3	485	0·6	13 [a]	9	3
Horse muscle	490	0·3	495	0·6	16—17 [1]	10—11	5
Human kidney	445	0·2	460	0·6	14 [b]	9	4
Human muscle	445	0·1	460	0·6 †	11 [a]	7	3
Mytilus edulis V	420	0·2	450	0·8	9 [f]	6	2
VI	445	0·1	480	1·0	13 [f]	8—9	3—4
Ox muscle	480	0·3	505	0·7 †	15 [1]	10	4
Rabbit liver VI	465	0·3	500	1·1	18 [e]	12	5
XII	460	0·2	500	1·0	17 [e]	9—10	6—7
XIII	470	0·3	480	1·0	15 [e]	9—10	4—5
Rabbit muscle II	460	0·2	490	0·9	11 [e]	6—7	3—4
Tetrahymena pyriformis I	445	0·3	465	0·9	13 [g]	8—9	3—4
II	440	0·3	485	0·9	14 [f]	9	4
Trichomonas foetus	445	0·4	500	1·1	15 [h]	11—12	2—3
Trichomonas gallinae II	440	0·3	480	1·0	13 [f]	8—9	3—4
Amylopectins:							
Malted barley	535	0·9	540	1·1	18 [i]	10—11	6—7
Potato III (Kerr's Pink)	540	1·1	545	0·7 †	22 [e]	16	5
IV (Epicure)	550	1·3	545	0·7 †	24 [j]	16	7
Protozoal (*Chilomonas paramecium*)	540	1·2	545	1·4	22 [k]	15—16	5—6
Waxy maize starch IV	530	1·1	560	1·6	22 [e]	15	6
Waxy sorghum starch I	540	1·1	560	1·2	25 [e]	15—16	8—9
β-Dextrins:							
Fœtal sheep liver glycogen	425	0·1	415	0·4 †	6—7	2—3	3
Mytilus edulis VI	420	0·03	420	0·3 †	7	2—3	3—4
Rabbit liver II	420	0·2	425	0·5 †	9	2—3	5—6
Waxy maize starch I	535	0·9	535	0·5 †	10	2—3	6—7

* See Footnotes to Table 1; additional references are, *g*, Manners and Ryley, *Biochem. J.*, 1952, **52**, 480; *h*, *idem*, *ibid.*, 1956, **59**, 369; *i*, Aspinall. Hirst, and McArthur, *J.*, 1955, 3075; *j*, Banks and Greenwood, unpublished work; *k*, Archibald, Hirst, Manners, and Ryley, *J.*, 1960, 556.

† Polysaccharide concentration 0·005%.

may enable a distinction to be made between a "glycogen"- and an "amylopectin"-type polysaccharide. The results obtained so far are in good agreement with those from potentiometric iodine titrations.[15]

Effect of Ammonium Sulphate on Polysaccharide–Iodine Colorations.—The exact colour of a polysaccharide–iodine solution depends upon many factors,[20] but at constant temperature and with a constant concentration of reactants it may be increased by the presence of various salts, especially ammonium sulphate.[21]

Schlamowitz examined the absorption spectra of several glycogen–iodine complexes in 50% saturated ammonium sulphate under conditions in which a large excess of glycogen was present.[22] For many glycogens, $\lambda_{max.}$ was *ca.* 496 mμ, and was independent of $\overline{CL}$, although $E_{max.}$ appeared to be roughly proportional to the $\overline{CL}$ value. The significance of these results is lessened by the fact that $\overline{CL}$ values were obtained [22] by a periodate oxidation method [23] which was originally applied to amylopectins. With glycogens, this method does not give satisfactory results (cf. Manners and Archibald [8]). Using Schlamowitz's conditions we measured $\lambda_{max.}$ and $E_{max.}$ for various glycogens, but were unable to find any relation between these properties and the $\overline{CL}$ (see Table 3).

In contrast to these results, addition of ammonium sulphate to 25% or 50% saturation in glycogen–iodine solutions, prepared as in Table 1, caused a marked increase in iodine-staining (Fig. 4, Table 4); *e.g.*, $E_{max.}$ values increased from 0·1 to 0·4 to the range 0·6—1·1. However, the solutions in 50% saturated ammonium sulphate became turbid, and a glycogen–iodine complex was slowly precipitated. (In one experiment, $E_{max.}$ fell from 0·87 to 0·82 within 15 minutes.) $E_{max.}$ values had, therefore, to be measured immediately after mixing. In later experiments, the stability of the solutions was increased by halving the glycogen concentration; this also halved $E_{max.}$ but did not affect $\lambda_{max.}$. In water or 25% ammonium sulphate solution, the glycogen–iodine solutions were clear and stable.

Glycogen β-dextrins were also examined; there was no appreciable change in $\lambda_{max.}$, although $E_{max.}$ increased (Table 4).

With amylopectin–iodine solutions, 50% ammonium sulphate caused only a slight increase in coloration; this was accounted for by an increase in $E_{max.}$ (from 0·9—1·2 to 1·1—1·6) rather than a change in $\lambda_{max.}$. Amylopectin β-dextrin behaved similarly.

Inspection of the results in Table 4 suggests that, with amylopectin and glycogen, $\lambda_{max.}$ is approximately related to the degree of branching; a correlation diagram is shown in Fig. 5. Since the length of the exterior chains is dependent on $\overline{CL}$, it follows that $\lambda_{max.}$ is also related to the exterior chain length. It may be possible to deduce $\overline{CL}$ values from measurements of $\lambda_{max.}$ under these conditions. By the method of least squares, with $\lambda_{max.}$ as the independent variable, these properties are related by the equation: $\overline{CL} = 16 + 0{\cdot}114\ (\lambda_{max.} - 500)$. The standard error in $\overline{CL}$ would be *ca.* 1·6 glucose residues. (We are indebted to Mr. A. G. Cock, Poultry Research Centre, Edinburgh, for this statistical analysis.)

It has been suggested [22] that ammonium sulphate facilitates iodine-complex formation by dehydration, providing a more hydrophobic environment for the iodine molecules. It is possible that a few of the longer B-chains in a glycogen molecule can, under these conditions, bind a limited amount of iodine by the helical mechanism rather than by adsorption.

Other salts, *e.g.*, magnesium sulphate, sodium sulphate, and sodium nitrate also increase the intensity of a glycogen–iodine coloration, but the effect is less than with ammonium sulphate.

[20] Morris, *J. Biol. Chem.*, 1946, **166**, 199.
[21] Sumner and Somers, *Arch. Biochem.*, 1944, **4**, 7.
[22] Schlamowitz, *J. Biol. Chem.*, 1951, **190**, 519.
[23] Potter and Hassid, *J. Amer. Chem. Soc.*, 1948, **70**, 3488.

Experimental

Most of the polysaccharide samples have been described elsewhere. We are grateful to Dr. G. O. Aspinall for the malt amylopectin, Dr. C. T. Greenwood for potato amylopectin IV, and Dr. J. R. Turvey for the *Zea mays* polysaccharides. The polysaccharide concentrations are based on the glucose content determined after acid-hydrolysis.

Effect of Polysaccharide Concentration on $\lambda_{max.}$.—Solutions containing severally 2·5, 1·5, and 1·0 mg. of horse muscle glycogen, 2·5 ml. of a 0·2% solution of iodine in 2·0% aqueous potassium iodide, and 1 drop of 3N-hydrochloric acid in a total volume of 25 ml. were prepared. In all three solutions, $\lambda_{max.}$ was 480 ± 5 mμ; $E_{max.}$ values were 0·25, 0·15, and 0·11 respectively.

Effect of Amylose as Impurity on Amylopectin–Iodine Solution.—Solutions containing potato amylopectin and 0, 5, or 10% w/w of potato amylose were prepared. The respective $\lambda_{max.}$ values were 555, 560, and 570 mμ, and $E_{max.}$ values 0·26, 0·28, and 0·33. The absorption of the amylose–iodine complexes alone was also measured. The effect was found to be additive.

Effect of β-Amylolysis on the Iodine-staining Power.—Digests containing rabbit liver VII glycogen and potato amylopectin (*ca.* 50 mg.) were incubated at pH 4·6 and 35° with barley β-amylase (2500 units) in a total volume of 50 ml. Samples (10 ml.) were removed at intervals, and heated to inactivate the enzyme. The conversion into maltose was determined, and equal *weights* of polysaccharide (≡ 2·3 mg.) stained with iodine and water or (≡ 1·15 mg.) stained with iodine and ammonium sulphate solution. Results are tabulated. If equal *volumes* of polysaccharide–β-amylase mixture are stained with iodine, $E_{max.}$ decreases as the percentage conversion into maltose increases.

Time of incubation (hr.)	β-Amylolysis limit (%)	Water		Aq. $(NH_4)_2SO_4$	
		$\lambda_{max.}$ (mμ)	$E_{max.}$	$\lambda_{max.}$ (mμ)	$E_{max.}$
		Potato amylopectin			
0	0	540	1·1	540	0·7
0·5	61	540	1·5	540	0·6
2·0	64	540	1·6	545	0·6
29	65	545	—	540	—
		Rabbit liver glycogen			
0	0	450	0·3	490	0·5
0·5	41	425	0·2	440	0·3
2·0	51	410	0·2	425	0·4
29	55	430	0·1	425	0·4

Effect of Various Salts on the Iodine-staining Power.—Rabbit muscle III or *Mytilus edulis* VI glycogen (final concentration 0·005%) was stained with iodine in the presence of half-saturated solutions of various salts, with the tabulated results.

	Rabbit muscle glycogen		*Mytilus edulis* glycogen			Rabbit muscle glycogen		*Mytilus edulis* glycogen	
Conditions	$\lambda_{max.}$ (mμ)	$E_{max.}$	$\lambda_{max.}$ (mμ)	$E_{max.}$	Conditions	$\lambda_{max.}$ (mμ)	$E_{max.}$	$\lambda_{max.}$ (mμ)	E_{max}
Water	495	0·30	445	0·12	K nitrate	470	0·34	—	—
NH_4 sulphate	490	0·96	455	0·73	K sulphate ...	485	0·31	445	0·25
NH_4 nitrate ...	505	0·22	445	0·13	Na nitrate ...	—	—	450	0·35
Ca chloride ...	—	—	430	0·27	Na sulphate ...	485	0·35	445	0·29
Mg sulphate ...	495	0·50	440	0·51					

The authors are indebted to Professor E. L. Hirst, C.B.E., F.R.S., for his interest and encouragement, to Mr. R. A. A. Hurst, Dr. I. G. Jones and Miss K. Shannon for assistance with preliminary experiments, to the Department of Scientific and Industrial Research for maintenance allowances (to A. R. A., I. D. F., A. M. L., and A. W.), and to Cerebos Ltd. for the award of a research scholarship (to G. M.).

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