Physicochemical Studies on Starches. Part III. The Interaction of Starches and Branched α-1 : 4-Glucosans with Iodine; and a Valve Microvoltmeter for Differential Potentiometric Titrations.*

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The uptake of iodine by 20 different starches has been studied by differential potentiometric titration, and iodine affinities for these samples are quoted. Contaminating protein alters significantly the apparent iodine affinity of starches. The iodine binding power of amylopectins, glycogens, and other branched α-1 : 4-glucosans has been studied. The differences observed, in conjunction with data for average unit-chain and external-chain lengths, suggest that variations in fine structure (*i.e.*, in degree of multiple branching) exist. In addition, details are given of a valve microvoltmeter developed to extend the scope and accuracy of differential potentiometric iodine titrations.

A QUANTITATIVE estimate of the amount of iodine bound by starch and its components can be obtained by the potentiometric-titration method introduced by Bates, French, and Rundle (*J. Amer. Chem. Soc.*, 1943, **65**, 142). Colorimetric methods developed subsequently (Hassid and McCready, *ibid.*, p. 1154; Bourne, Haworth, Macey, and Peat, *J.*, 1948, 924) are more arbitrary, although useful for comparative measurements. The optical absorption characteristics of the iodine complexes of different amyloses and amylopectins vary (cf. Baldwin, Bear, and Rundle, *J. Amer. Chem. Soc.*, 1944, **66**, 111; Kerr, Cleveland, and Katzbeck, *ibid.*, 1951, **73**, 3916), and the measurements are not absolute or capable of the same accuracy, particularly for amylopectin (cf. Higginbotham and Morrison, *Shirley Inst. Mem.*, 1948, **22**, 141).

Bates and his co-workers (*loc. cit.*) measured the potential between a bright platinum electrode in the starch–iodine–iodide solution and a standard calomel electrode, and thus were able to calculate the equilibrum concentration of free iodine in the mixture. However, the elegant *differential* method of Gilbert and Marriott (*Trans. Faraday Soc.*, 1948, **44**, 84) is much more satisfactory for accurate work at the necessary low free-iodine concentrations. In this technique, the starch–iodine–iodide solution and the blank–iodine–iodide solution are arranged as opposing half-cells connected by a salt bridge; the equilibrium free-iodine concentration in the starch solution can then be found directly, and separate titrations for reagent blanks are not required. As has recently been mentioned (Mould, *Biochem. J.*, 1954, **58**, 593), the scope of the differential technique and the accuracy and reproducibility of results obtained by its use depend on the availability of a null-potential indicator of great zero stability combined with high sensitivity. Such an electronic device, providing high sensitivity (30 mm./mv) and zero stability has already been described (Anderson and Greenwood, *Chem. and Ind.*, 1953, 476). This electrometer gives excellent results for

* Part II, *J.*, 1955, 225.

routine analyses of unfractionated starches, but reliable readings are not possible when the free-iodine concentrations exceed 8×10^{-6}M. The logarithmic decrease in possible sensitivity with increasing free-iodine concentration in both half-cells is an inherent disadvantage of the differential-titration technique. Nevertheless, a ten-fold increase in sensitivity was sought, to permit an accurate study of the iodine-binding power of branched α-1 : 4-glucosans (*e.g.*, the amylopectin component of starch) on which relatively little work has yet been carried out. It appeared possible that such a study could give some details of fine structure.

Mikus, Hixon, and Rundle (*J. Amer. Chem. Soc.*, 1946, **68**, 1115) consider that the low iodine-binding power of branched α-1 : 4-glucosans is inexplicable in terms of hydrogen bonding (cf. Whistler and Hilbert, *ibid.*, 1945, **67**, 1161). They suggested that the large number of branch-points prevents helix formation and decreases the dipolar forces thought to be responsible for complex formation between iodine and the amylose component of starch. [Higginbotham (*Shirley Inst. Mem.*, 1949, **23**, 171) has suggested that, in amylopectin, adsorption of I_2 and I_3^- occurs in addition to complex formation in helices.] The amount of helix formation possible, and hence the iodine uptake, must be related to fine structure. The several model structures proposed for amylopectin [*i.e.*, the " laminated " structure (Haworth, Hirst, and Isherwood, *J.*, 1937, 577; Halsall, Hirst, and Jones, *J.*, 1949, 3200), the " herring-bone " structure (Staudinger and Eilers, *Annalen*, 1937, **527**, 195), and the " ramified " structure (Meyer and Bernfeld, *Helv. Chim. Acta*, 1940, **23**, 857)] all contain different arrangements of the same linear basic chains (Myrbäck and Sillén, *Acta Chem. Scand.*, 1949, **3**, 190), which Peat, Whelan, and Thomas (*J.*, 1952, 4546) have suggested be termed A-, B-, and C-chains. The three models differ, therefore, only in their ratio of A : B chains, *i.e.*, in the *degree of multiple branching*. Similar considerations also apply to other branched α-1 : 4-glucosans. Variations in fine structure must exist to explain the difference in limiting viscosity numbers of the two branched glucosans, amylopectin and glycogen (cf. Greenwood and Robertson, *J.*, 1954, 3769). Any method which can give further indications of differences in fine structure is important.

Experimental

Details of Valve Microvoltmeter.—The few valve millivoltmeters described in recent years (see, *e.g.*, Morton, *Trans. Faraday Soc.*, 1948, **44**, 588; Gray, *Discuss. Faraday Soc.*, 1950, **8**, 331, and personal communication; Scroggie, *Wireless World*, 1952, **14**; Furman, *Analyt. Chem.*, 1954, **26**, 84) were found either to be incapable of modification for our purpose, or, when constructed, had a zero-drift about 100 times greater than required.

Attempts to improve the sensitivity of the original circuit (Anderson and Greenwood, *loc. cit.*) by using miniature valves of high mutual conductance ($g_m = 10$) with 22-v heaters (run from the stabilized high-tension supply) were unsuccessful as the valves would not function under these " under-run " conditions. The desired standard was finally achieved by improving the stability of both high- and low-tension voltage supplies, then amplifying the output by a matched pair of valves functioning as a cathode-coupled amplifier. This design had the advantage of retaining the satisfactory high input impedance and low grid current of the original circuit, and moreover was still simple, depending on fundamental balance of valves and components rather than compensating, and therefore complicating, circuitry. The final circuit is shown in Fig. 1*b*. Very accurate readings can be made up to free-iodine concentrations of 10^{-5}M.

Fig. 1*a* shows how a harmonic-filtered constant-voltage transformer supplies the input voltage to an Ediswan stabilized power unit (Type R1095) and to an accumulator trickle-charger. The latter charges a pile of 12 × 2-v cells (arranged in series/parallel to give 4-v and 6-v outputs) at the same currents as are being taken by the two pairs of valve filaments. A highly insulated switch allows the galvanometer (sensitivity 109 mm./microamp., internal resistance 402 ohm) to be connected either between K_1 and K_2 (so giving the original circuit; sensitivity = 30 mm./mv) or between A_3 and A_4, which gives a sensitivity of 315 mm./mv. In conjunction with the low rate of zero drift attained, this permits potentials of 1 microvolt to be measured. The circuit is extremely stable towards external electrical interferences, since both pairs of valves have been selected *under actual operating conditions* as the best matched pairs obtainable from a large number. The operating conditions of both pairs differ, and are to some extent interdependent; the choice of individual valves to form pairs and of optimum values

for the resistors could only be made by continued " refinements." Wire-wound resistors, matched in pairs to within 1%, are used throughout, and all grid leads are of screened-type coaxial cable. For best results, the electrometer must be adequately protected against vibration, mechanical shock, and local changes in room temperature. The value of R_6 giving the optimum ratio of sensitivity to stability is 330 ohms; decreasing this value gives increased sensitivity, but the circuit may then tend to oscillate, creating instability. Although this can be minimized by inserting " grid-stopper " resistors (47,000 ohms; 0·5 w) inside the grid top-caps of V_3 and V_4, the thermal effects associated with these resistors contributed to zero drift. The introduction of negative feed-back, either by connecting V_3A to V_1G, and V_4A to V_2G, *via* 1 megohm resistors, or by cross-connecting V_4A to V_3Sc and V_3A to V_4Sc, reduced rather than improved stability, and it was shown that zero drift is largely due to fluctuations in the low-tension, and not in the high-tension, supply. Absolute matching of V_3 and V_4 is achieved by connecting both V_3 and V_4 grids to V_1K (with V_1G to V_1K *via* a 2-megohm resistor), and, with P_4 pre-set at its optimum value, P_3 is adjusted so that the galvanometer deflection when connected across A_3/A_4 is zero. After V_4G has been returned to V_2K, P_3 is never altered, and all zeroing adjustments are made by using the " set zero " coarse and fine controls for both A_3/A_4 and K_1/K_2 systems.

FIG. 1(*a*) *and* (*b*).

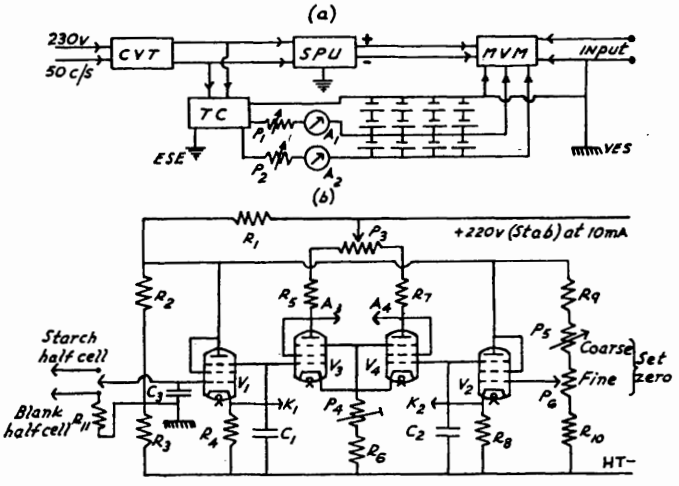

FIG. 1(*a*). *Block diagram of circuit.*

CVT, Constant voltage transformer. SPU, Stabilized power unit. MVM, Microvoltmeter. TC, Trickle-charger. ESE, Electricity supply earth. VES, Virtual earth system. A_1, *Ammeter (set at* 0·88 *amp.).* A_2, *Ammeter (set at* 1·25 *amp.).* P_1, 15 Ω/5 *amp.* P_2, 12 Ω/7 *amp.*

FIG. 1(*b*). *Microvoltmeter circuit.*

C_1, C_2, 0·01 μF, *mica.* C_3, 0·1 μF, *mica.* P_3, 500 Ω; P_4, 250 Ω; P_5, 10 *K* (*all* 5 w). P_6, 10Ω/2 w. (*All linear, wire-wound.*)

R_1, 100 *K*; R_2, 68 *K*; R_3, R_{10}, 500 Ω; R_4, R_8, 10 *K*; R_5, R_7, 22 *K*; R_6, 250 Ω; R_9, 65 *K*. (*All* 5 w, *wire-wound.*)

R_{11}, 2*M*, 1 w, *carbon.*

V_1, V_2 = VR 116; V_3, V_4 = VR 65 (SP61); V_1 *and* V_2, *heaters* 4 v *at* 0·88 *amp.*; V_A, 50 v; V_G, 0 v, V_K, 1 v; V_3 *and* V_4, *heaters* 6 v *at* 1·25 *amp.*; V_A, 110 v; V_G, 1 v; V_K, 2·4 v.

The two-way switch shown in the input circuit to V_1 (Fig. 1*b*) must be very highly insulated and must make-before-break so that the grid of V_1 is never on open circuit. A satisfactory switch was made from a thick block of paraffin wax containing pools of mercury, between which contact was made by a tilting copper-wire framework. The inter-electrode resistance is approximately 2 megohms; when the zero-reading of the electrometer is being checked, the grid of V_1 is therefore returned to earth *via* R_{11} so that the operating conditions of V_1 are changed as little as possible.

Details of Titration Cells.—The titration cells (1-l. Pyrex flasks) and salt bridge were similar to Gilbert and Marriott's (*loc. cit.*), except that stirring was automatic and continuous. Additions of iodine were made *via* additional necks in each flask. All four necks were fitted with ground-glass joints, enabling the apparatus to be completely sealed, stirring being made

through Quickfit stirrer glands. [No loss of iodine occurred through volatilization in the time taken for titration (*i.e.*, 40 min.).] The electrodes consisted of platinum foil (2 × 2 cm.). By careful strain-free construction and thorough cleaning, it was possible to obtain a pair of electrodes between which no potential difference existed when placed in the same solution of electrolyte.

Reagents.—All reagents were of "AnalaR" grade, used without further purification.

Preparation of Starch Solutions.—All samples were exhaustively extracted with boiling methanol to remove traces of fat. This is essential. For example, an undefatted sample of commercial rice starch apparently contained 11·8% of amylose, but after being refluxed with methanol (5 treatments; each of 3 hr.) a constant value of 18·5% of amylose was given (7·3% of the original weight of starch was extracted by the methanol). After removal of fat, samples were dried *in vacuo* at 80° for several hours before being weighed by means of a stoppered weighing-stick into a graduated flask. Suitable weights for titration were: starch, 10 mg.; amylopectin and glycogen, 30 mg. Dissolution was achieved by shaking the starch overnight at room temperature with 0·2M-potassium hydroxide (10 ml.) after moistening it with ethanol (2 drops). In certain cases it was necessary to heat the mixture at 95° for 3 min. before shaking. [The effect of pretreatment of whole starch with alkali was investigated as the amylose component degrades in this solvent (Bottle, Gilbert, Greenwood, and Saad, *Chem. and Ind.*, 1953, 541). Ageing at room temperature had no effect, and heating a starch in 0·2M- and 1M-potassium hydroxide for 30 min. at 95° had a negligible effect on the iodine affinity of the sample.] Immediately before addition to the titration half-cell, the alkaline polysaccharide solutions were brought to pH 5·85 by the addition of a predetermined volume of 0·4N-phosphoric acid. A blank solution containing no starch was similarly prepared.

Titration Conditions and Procedure.—Titration conditions were: [iodide], 0·01M; pH, 5·85; temp., 20°. 0·01M-Iodide was chosen so that the addition of 0·01M-iodine–potassium iodide did not alter the iodide concentration in the half-cells during a titration, and thus corrections such as applied by Mould (*loc. cit.*) were avoided. The electrolyte solution (2 l.) contained 0·1M-potassium iodide (210 ml.) and M/15-phosphate buffer (15 ml.; pH, 5·85). This solution (800 ml.) was placed in each half-cell, and stirred for 30 min. in the thermostat to allow for temperature equilibrium. The neutralized polysaccharide and blank solutions were then added to their respective half-cells and the standard flasks carefully rinsed, giving a total volume of 840 ml. (*i.e.*, [iodide], 0·01M). With careful preparation of all solutions, and with temperature equilibration, no significant off-balance potentials existed at the start of titrations, and the "depolarizing" procedure described by Gilbert and Hybart (see Gilbert, Greenwood, and Hybart, *J.*, 1954, 4454) was not necessary. Points on the titration curve were obtained by adding small increments of 0·01M-iodine–potassium iodide by means of an "Agla" micrometer syringe to the solution cell, then adding the same iodine solution to the control until the concentration of free iodine in each was identical, after 2—5 min. (or longer for branched glucosans) had been allowed for equilibration. The difference between the volume of iodine added to the solution cell and that added to the control gave the amount of iodine bound by the starch, the iodide concentrations in each cell being identical. The total free iodine in the starch solution (*i.e.*, $I_2 + I_3^-$) was plotted against mg. of iodine bound per 100 mg. of polysaccharide.

Reproducibility of Technique.—Results were independent of the time taken to complete a titration curve (provided true equilibration had been achieved at each free iodine concentration), and also of the sample weight. The reproducibility is within ±2% of the iodine affinity for an unfractionated starch (*i.e.*, for a starch having an iodine affinity of 5·0%, the results of 6 determinations lay between 4·9 and 5·1%).

As described by Gilbert and Hybart (*loc. cit.*), addition of excess of thiosulphate enabled the titration curve for any starch sample to be repeated. When the titration was repeated at 24- or 48-hr. intervals for 14 days, the starch solution being left in contact with iodine throughout, the observed changes in iodine affinity could be attributed to retrogradation of the amylose component. For waxy maize starch, the titration curve was unaltered after the sample had been in contact with iodine for 17 days. Similarly, for rabbit-liver glycogen, the curve was unaltered after contact with iodine for 21 and 31 days.

Results and Discussion

Fig. 2 shows some typical titration curves for starch samples obtained by plotting the amount of bound iodine against the total free-iodine concentration. Each starch was characterized by its "iodine affinity," which is a measure of the preferential uptake of

iodine by the linear amylose component. At the free-iodine concentration saturating the amylose, the amount of iodine bound by the amylopectin is not negligible (see Anderson and Greenwood, *Chem. and Ind.*, 1954, 642, and below). An estimate of the amount of iodine bound by the amylose component was therefore obtained by extrapolating the linear portion of the titration curve to zero free-iodine concentration. All iodine affinities quoted here were calculated on this basis. The percentage of amylose in a starch can be calculated from this value when the corresponding value for pure amylose is known (Bates and his co-workers, *loc. cit.*). However, as previously mentioned (Greenwood and Robertson, *loc. cit.*), the only accurate method of doing this involves experimental determination of the maximum iodine-binding power of the *pure* amylose component of the starch under examination. Hence, when it is not desired to fractionate any starch exhaustively, it is more satisfactory to quote its iodine affinity, rather than an arbitrary percentage of amylose

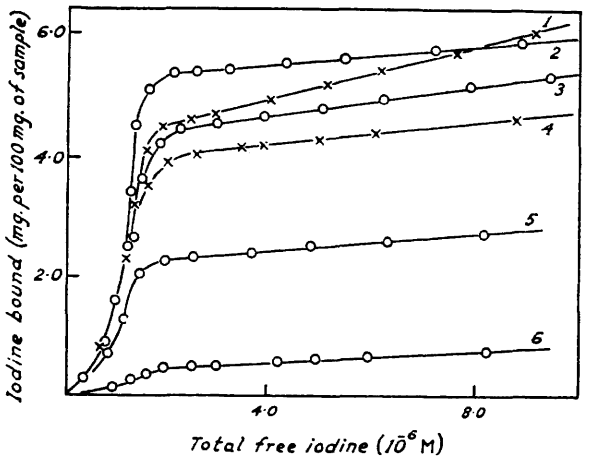

FIG. 2. *Typical iodine-titration curves for starches.*
1, *Potato* (*Golden Wonder*). 2, Iris germanica. 3, *Barley II.* 4, Hevea brasiliensis. 5, *Parsnip.* 6, *Waxy maize.*

(cf. Schoch in Radley, "Starch and its Derivatives," Chapman and Hall, London, 1953, Vol. 1, p. 123). For comparison with other workers' results, however, percentages of amylose have been calculated, using 19·2% of bound iodine as the maximum iodine-binding power of pure amylose under our experimental conditions (Greenwood and Robertson, *loc. cit.*).

Table 1 summarizes the results for starch samples. It is of interest that starches from different varieties of the same botanical source may show variation in the apparent percentage of amylose; this is in agreement with Doremur, Creshaw, and Thurber's results (*Cereal Chem.*, 1951, **28**, 308). The slope of the linear portion of the titration curve for all the potato starches studied was considerably greater than for other starches.

Contaminants affect the amount of iodine bound by a starch. Interference by fatty acids is well known (cf. Mikus, Hixon, and Rundle, *loc. cit.*). In addition, protein has now been found to have considerable effect (see Table 2), and its presence causes distortion of the titration curve. For oat starch, the effect is to increase the apparent binding power; the protein removes free iodine from solution, and estimates of the true affinity are best obtained by *not* correcting for the percentage of protein present. However, for rubber seed and pea starches, and for synthetic mixtures of protein with potato starch, the protein apparently suppresses starch–iodine complex formation. Interference by protein has also been found during the study of protozoal starches (unpublished observations). It is therefore essential to remove contaminating protein before titrations are attempted.

As briefly reported (Anderson and Greenwood, *loc. cit.*), the difference in iodine-binding power of normal amylopectins and glycogens is sufficiently large to characterize these two structure types. The iodine-binding power of these materials must be fundamentally related to differences in the average length of unit chain, degree of multiple branching, and external-chain length. For a group of polysaccharides having a similar degree of

TABLE 1. *The iodine-binding power of starches.*

Source of starch *	No. of detns.	Iodine affinity †	Amylose (%) ‡	Slope of linear portion of curve §
Arrowroot	4	3·94	20·5	0·13
Banana	3	3·23	16·8	0·10
Barley I	2	4·22	22·0	0·12
Barley II	4	4·22	22·0	0·12
Hevea brasiliensis seed	3	3·86	20·0	0·10
Iris germanica	3	5·18	27·0	0·09
Maize	3	4·61	24·0	0·11
Oat I	2	5·00	26·0	0·13
Oat II	7	5·00	26·0	0·13
Parsnip	4	2·13	11·1	0·08
Pearl manioc	2	3·02	15·7	0·07
Potato I	7	3·94	20·4	0·28
Potato II	4	4·03	21·0	0·24
Potato III	2	4·03	21·0	0·23
Rice	6	3·55	18·5	0·09
Sago	4	5·00	26·0	0·08
Sweet potato	2	3·42	17·8	0·27
Tapioca	4	3·21	16·7	0·07
Waxy maize	5	0·27	1·4	0·06
Wheat	6	4·80	25·0	0·05

* Origin of samples as in Part II of this series (*loc. cit.*), except for *Hevea brasiliensis* seed (Greenwood and Robertson, *loc. cit.*).

† Expressed as mg. of iodine bound per 100 mg. of starch.

‡ Calc. as iodine affinity ÷ 19·2.

§ Expressed as % of iodine bound per total free-iodine concn. × 10^6(M) [range of total free iodine (2—10) × 10^{-6}M].

TABLE 2. *The effect of contaminating protein on the iodine-binding power of starches.*

Starch sample	Protein (%) *	No. of detns.	Iodine affinity † Sample wt. uncorr.	Iodine affinity † Sample wt. corr. for protein
Hevea brasiliensis seed A [a]	0·31	3	—	3·86
Hevea brasiliensis seed B	2·56	2	—	3·01
Oat II (*a*) [b]	0·19	6	5·00	5·00
,, II (*b*)	0·45	3	5·44	5·48
,, II (*c*)	2·94	3	5·97	6·14
,, II (*d*)	22·7	3	5·27	6·80
,, II (*e*)	45·6	2	4·78	8·80
Pea (proteinaceous) [c]	37·5	2	—	9·20
Pea (chloral hydrate extracted)	4·93	1	—	15·1
Potato I	0	7	—	3·94
,, + edestin	2·0	1	—	3·78
,, ,,	10·0	1	—	3·05
,, + egg albumin	2·5	1	—	3·66
,, ,,	16·5	1	—	2·93
,, + tyrosine	11·0	1	—	3·45

* % of N, × 6·25.

† Expressed as mg. of iodine bound per 100 mg. of starch.

[a] Greenwood and Robertson, *loc. cit.* [b] Anderson and Greenwood, *J. Sci. Food Agric.*, in the press. [c] Sample kindly provided by Dr. E. J. Bourne; see *Nature*, 1948, **161**, 206.

branching, it is also probable that variations in the amount of iodine bound are related to the length of *external chain* available for helix formation.

Titrations of different amylopectin samples have always shown evidence of preferential uptake of iodine by linear material. To compare iodine-binding powers, therefore, such preferential uptake has been corrected for by extrapolating the titration curve to zero free-iodine concentration, with this extrapolated point being taken as the origin for the iodine-binding curve. [This preferential uptake was presumably due to contaminating amylose; this is extremely difficult to remove (cf. Gilbert, Greenwood, and Hybart, *loc. cit.*), and the presence of some long branches in the amylopectin cannot be entirely excluded (cf. Swanson, *J. Biol. Chem.*, 1948, **172**, 825).] In all the glycogen samples so far examined there was no evidence of preferential uptake.

Fig. 3 and Table 3 show the results for some amylopectins and glycogens. In the range

of concentrations employed, the amount of iodine bound is directly proportional, within experimental error, to the total free-iodine concentration. This would be expected if the iodine is bound as a co-linear core of iodine and tri-iodide molecules arranged end-to-end in the available helices. One iodine molecule can be accommodated in a helix of about six glucose units (Baldwin, Bear, and Rundle, *J. Amer. Chem. Soc.*, 1944, **66**, 111). Since the length of external chain available for helix formation is only 14—18 glucose units (*i.e.*, about three helices) for amylopectins, and 8—11 glucose residues (*i.e.*, 1—2 helices) for glycogen (cf. Manners, *loc. cit.*), the amount of iodine-binding possible is small.

Higginbotham (*loc. cit.*) has suggested that *adsorption* of iodine molecules (or tri-iodide ions) may also occur. However, at the low iodine concentrations used here, adsorption effects would be small, and are indeed unlikely to occur in view of the negligible effect on the titration curves of increased polysaccharide concentrations.

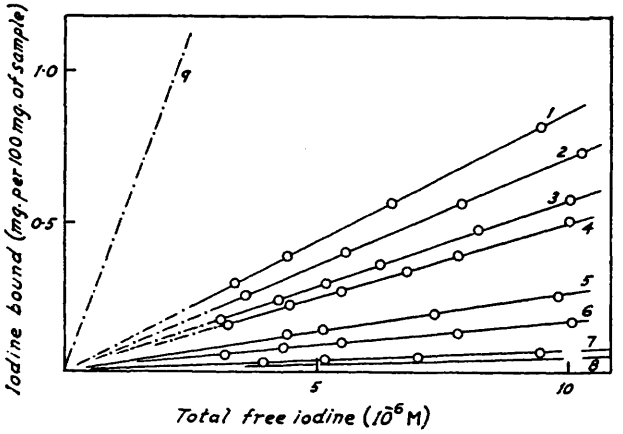

FIG. 3. *The iodine-binding power of branched* α-1 : 4-*glucosans.*

1, *Barley II amylopectin.* 2, Hevea brasiliensis *seed amylopectin.* 3, *Waxy maize starch.* 4, *Oat I amylopectin.* 5, *Glycogen* (*chain-length* 18 *glucose units*). 6, *Zea mays polysaccharides.* 7, Tetrahymena pyriformis *polysaccharide.* 8, *Rabbit-liver glycogen.* 9, *Wrinkled-pea amylopectin.*

Fig. 3 shows that waxy maize starch behaves as a typical amylopectin, and the polysaccharide from the ciliate *Tetrahymena pyriformis* (Manners and Ryley, *Biochem. J.*, 1952, **52**, 480) as a glycogen. Molecular weight must be of minor importance. For example,

TABLE 3. *The iodine-binding power of branched* α-1 : 4-*glucosans.*

Sample	Linear material (%)	Slope of titration curve *	Av. length of unit chain	Length of external chain †
Barley II amylopectin	2·6	0·090	23 [a]	16 [g]
Hevea brasiliensis amylopectin	0·8	0·074	23 [a]	—
Oat I amylopectin	3·2	0·052	20·3 [a]	—
Ascaris lumbricoides glycogen	—	0·009	12 [b]	—
Rabbit-liver glycogen	—	0·006	13 [b]	8 [b]
Tetrahymena pyriformis polysaccharide	—	0·007	13 [b]	8—9 [b]
Waxy maize starch	1·4	0·060	20 [a]	15—16 [b]
Rabbit-liver glycogen	—	0·028	18 [c]	12
Wrinkled-pea amylopectin	3·4	0·485	36 [d]	—
Zea mays polysaccharide (insoluble)	0·26	0·019	12 [e]	8
,, ,, ,,	0·6	0·017	13 [f]	9
,, ,, (soluble)	0·6	0·007	11	7

* Expressed as for Table 2. † No. of glucose units removed on β-amylolysis +2·5.

[a] Part II, *loc. cit.*; [b] Manners, *Ann. Reports*, 1953, **50**, 288; [c] Haworth, Hirst, and Isherwood, *J.*, 1937, 377; [d] Potter, Silveira, McCready, and Owens, *J. Amer. Chem. Soc.*, 1953, **75**, 1335; [e] Dvonch and Whistler, *J. Biol. Chem.*, 1949, **181**, 889; [f] Dr. W. J. Whelan, personal communication; [g] Aspinall, Hirst, and McArthur, *J.*, 1955, in the press.

the amylopectin from rubber-seed starch (D.P. 6000) binds about ten times more iodine than rabbit-liver glycogen (D.P. 30,000). (For values of D.P., see Greenwood and Robertson, *loc. cit.*)

It was possible to test the hypothesis that fine structure governed iodine-uptake when samples of " abnormal " branched α-1 : 4-glucosans became available. Samples of the

water-soluble polysaccharides from sweet corn (*Zea mays*) were kindly placed at our disposal by Drs. R. L. Whistler and W. J. Whelan. The exact structural nature of these polysaccharides has been in dispute (cf. Morris and Morris, *J. Biol. Chem.*, 1939, **130**, 535; Hassid and McCready, *J. Amer. Chem Soc.*, 1941, **63**, 1132; Sumner and Summers, *Arch. Biochem.*, 1944, **4**, 7; Cameron, *Genetics*, 1947, **32**, 459; Dvonch and Whistler, *J. Biol. Chem., loc. cit.*). Whilst these materials have an average length of unit chain of 12—13 and 10—11 glucose residues respectively (*idem, loc. cit.*; Whelan, personal communication), the iodine-uptake was three to four times greater than that for a glycogen of corresponding average chain length (see Table 3), although the molecular weights were of the same order (Greenwood, unpublished work). It is, therefore, suggested that these polysaccharides have a degree of multiple branching *intermediate* between those of glycogen and amylopectin, and are therefore neither in the one class nor the other. [It is of interest that Wolff, Watson, and Rist (*J. Amer. Chem. Soc.*, 1953, **75**, 4897) reached a similar conclusion from a study of the tricarbanilates of polyglucosans with different linkages.]

An abnormal rabbit-liver glycogen [shown by Haworth, Hirst, and Isherwood (*loc. cit.*) from methylation studies to have an average unit chain of 18 glucose residues] bound about five times more iodine than a normal glycogen and appeared to behave more as an amylopectin-type structure. Without additional information, it is not possible to say whether this is due to the increased average length of the external chains (*i.e.*, 12 residues) or to a variation in the degree of branching. [The abnormal character of this glycogen has been confirmed by Professor F. Smith (personal communication), who found it to possess an abnormal precipitin reaction with concanavalin-A.]

The amylopectin from wrinkled-pea starch (*var.* Perfection) has been shown to possess abnormal iodine-binding power, and an average unit chain of 36 glucose residues (Potter, Silveira, McCready, and Owens, *loc. cit.*). These authors deduced from spot tests that no amylose was present. A sample of this material, kindly provided by Dr. R. M. McCready, gave an abnormal titration curve indicating the presence of some linear material. The iodine-binding power was about six times greater than that for a normal amylopectin (Table 3).

Although data regarding the average external chain length of the amylopectins studied are not complete, the results quoted in Table 3 suggest that small variations may exist in the degree of multiple branching of this group of polysaccharides. Additional evidence might be obtained from a study of the appropriate limit dextrins (cf. Foster and Smith, *Iowa State Coll. J. Sci.*, 1953, **27**, 467), and experiments on these lines are in progress. Similar effects have been found from a study of a large number of glycogen samples (Greenwood and Manners, unpublished work).

Hence, potentiometric determinations of the amount of iodine bound by branched α-1 : 4-glucosans, in conjunction with estimations of chain length, should enable an estimate of the degree of multiple branching to be obtained if the method can be confirmed by enzymic degradative experiments (Peat and his co-workers, *loc. cit.*; Hirst and Manners, *Chem. and Ind.*, 1954, 224). Such experiments are now in progress in these laboratories.

It is of interest that, for the samples studied, the slope of the linear portion of the titration curve for a whole starch is greater than that for the corresponding isolated amylopectin. This implies that sub-fractionation has occurred, and emphasizes the importance of study of all supernatant and precipitated materials obtained during fractionation (cf. Greenwood and Robertson, *loc. cit.*).

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