[CONTRIBUTION FROM THE GEORGE M. MOFFETT RESEARCH LABORATORIES, CORN PRODUCTS REFINING COMPANY]

The Molecular Magnitude of Amylopectin

BY RALPH W. KERR, F. C. CLEVELAND AND W. J. KATZBECK

Although the order of magnitude for the molecular weight of the amyloses appears to have been established in recent years, considerable difference of opinion still exists concerning the average size of amylopectin molecules. In general, chemical methods give comparatively low DP_n values whereas physical measurements indicate very much higher values. Thus, for example, Meyer and co-workers[1] have reported DP_n values of 280 for corn amylopectin and 1100 for potato amylopectin, using an end-group oxidation method with dinitrosalicylate. On the other hand, physical measurements have given DP_n values ranging from 1450 for potato amylopectin by osmotic pressure determinations[2] and 6000 by ultracentrifugal studies[3] to as high as 20,000 to 50,000 for corn amylopectin using the ultracentrifuge.[4] Intermediate values to these extremes have been reported.[5,6,7]

There are obvious possible explanations to account for the great discrepancy in molecular weight values. First of all, in chemical methods the amylopectin may be degraded during the test more than the control of known molecular weight with which it is being compared. Secondly, in physical testing of solutions, the amylopectin preparations may not disperse molecularly, and the measurement may be one of particle weight or possibly both degradation and association effects contribute simultaneously to the discrepancy. It is very difficult to explore experimentally either of these possibilities. However, bearing in mind the obvious obstacles which have been mentioned the problem of molecular weight determination of amylopectin has been approached by several lines of attack. Inasmuch as the values obtained by all of these approaches now tend to converge into a relatively narrow range, it is believed that some assurance is provided that the values are at least of the correct order of magnitude for the true molecular weight.

Four methods have been used in the present investigation: (a) direct determination of the molecular weights of corn and tapioca amylopectins by osmotic pressure measurements on their acetates prepared by a recently reported[8] derivatization method; (b) calculation of the DP_n of corn amylopectin from the yield and osmotically determined DP_n of its β-amylase limit dextrin[9]; (c) calculation of the DP_n of corn amylopectin from the DP_n and composition of whole corn starch; and (d) calculations of the DP_n of amylopectin from relationships found between DP_n values and chemical and physical properties of a series of hydrolyzed B-fractions and amylopectin subfractions of known molecular weight. The latter studies have consisted of alkali lability measurements, oxidation by alkaline ferricyanide and by dinitrosalicylate, and viscosity determinations.

Experimental Section

Determination of Osmotic Pressures.—Osmotic pressures were determined on chloroform solutions of the carbohydrate triacetates according to the general procedures given previously[9,10] using a Fuoss–Mead cell and uncoated cellophane membranes. The triacetates were prepared by the formamide dispersion method with acetic anhydride and pyridine as catalyst. This method has also been given in detail.[9] Acetyl values were in all cases within the limits 44.6 to 44.8%.

In the measurement of very small osmotic pressures it was again found that the static method was more suitable. In addition, in these experiments it was found necessary to determine the zero point with solvent alone every time the cell was set up. These observations extended over several days time. A new membrane was used for each material tested and if the zero point of the cell was found to exceed ± 0.2 mm. of chloroform, the membrane was discarded and the cell set up anew.

The lowest concentrations were used first and ordinarily these were 0.1 g. per 100 ml. The solution side of the cell was flushed with this concentration of solution over a period of several days time, until the osmotic pressure came to constant value. Initial values were usually low. In one experimental run with corn amylopectin acetate after the highest concentration had been used, the cell was washed extensively with chloroform and dilute solutions were reemployed giving satisfactory check results.

In order to establish with greater certainty the anomalous shape of osmotic pressure curves obtained with B-fraction acetates in chloroform, particularly the initial negative slope, one preparation, the 80 F-B fraction acetate, which gave 1.8 to 1.95 mm. of pressure at a concentration of 0.1 g. per 100 ml., was also run at 0.05 g. per 100 ml. On two runs, values of 1.1 and 1.0 mm. pressure were obtained as indicated by the results in the Table II thus confirming the negative slope in the osmotic pressure–concentration curve as infinite dilution is approached.

The method used for the determination of alkali number was that of Schoch and Jensen.[11] Intrinsic viscosity in N KOH at 35° was determined according to procedures given by Lansky, Kooi and Schoch.[12] Cleveland and Kerr[13] have outlined the method for the determination of ferricyanide number. Furthermore this test as well as the others mentioned are described in some detail by Kerr.[14]

Dinitrosalicylate color value was determined as follows: Eastman Kodak Co. 3,5-dinitrosalicylic acid was twice recrystallized and 666 mg. dissolved in 200 ml. of 1 N KOH. Exactly one-half gram dry basis of starch fraction was dissolved in 15 ml. of the reagent solution, with intermittent stirring over a period of one hour at 25° in a test-tube 6 × 0.75 inches. A blank was prepared containing only 15 ml. of reagent.

The tubes were immersed in a water-bath at 65° and held for 60 minutes. They were immediately cooled and the contents made up to 50 ml. in volumetric flasks with water.

(1) K. H. Meyer, P. Bernfeld, R. A. Boissonnas, P. Gurtler and G. Noelting, *J. Phys. Coll. Chem.*, **53**, 319 (1949).

(2) M. Samec, *Z. physiol. Chem.*, **259**, 204 (1939); **267**, 243 (1941).

(3) F. E. Horan, Dissertation, Columbia University, New York, 1944.

(4) T. G. Fox, Dissertation, Columbia University, New York, 1943.

(5) C. O. Beckmann and Q. Landis, THIS JOURNAL, **61**, 1495, 1504 (1939).

(6) J. S. Coles, Dissertation, Columbia University, New York, 1941.

(7) A. L. Potter and W. Z. Hassid, THIS JOURNAL, **70**, 3744 (1948).

(8) J. F. Carson and W. D. Maclay, *ibid.*, **68**, 1015 (1946).

(9) R. W. Kerr and F. C. Cleveland, *ibid.*, **71**, 3455 (1949).

(10) F. C. Cleveland and R. W. Kerr, *ibid.*, **71**, 16 (1949).

(11) T. J. Schoch and C. C. Jensen, *Ind. Eng. Chem., Anal. Ed.*, **12**, 531 (1940).

(12) S. Lansky, M. Kooi and T. J. Schoch, THIS JOURNAL, **71**, 4066 (1949).

(13) F. C. Cleveland and R. W. Kerr, *Cereal Chem.*, **25**, 133 (1948).

(14) R. W. Kerr, "Chemistry and Industry of Starch," Academic Press, New York, N. Y., Chapt. XXIV, 1950.

Per cent. light transmission values were determined using 40-mm. cells in a Coleman Spectrophotometer Model 14 at a wave length of 500 mμ and setting the instrument at 100% light transmission when the blank reagent test solution was used.

Turbidity was noted in some of the starch fraction tests and this was corrected for by determination of the loss in per cent. light transmission observed, compared to the blank, at a wave length of 750 mμ.

Starch fractionation was performed on methanol defatted starch samples using Pentasol, the agent preferred by Lansky, Kooi and Schoch[12] to precipitate the A-fraction from autoclaved aqueous sols. The B-fractions were recovered from the centrifugates by adding methanol.

The series of acid hydrolyzed corn starches employed are samples of commercial thin boiling starches at various commercial fluidity grades. Measurement of fluidity has been described.[14] These products are made by heating corn starch in an aqueous solution of 0.1 to 0.15 N H_2SO_4 at 50° for appropriate lengths of time, neutralizing with sodium carbonate, washing and drying. The exception is the 90 F-starch which is made by impregnating a wet filter cake with dilute HCl, drying at a temperature less than the gelatinization point, and neutralizing with ammonia.

Discussion

Our preliminary studies on the measurement of the osmotic pressure of amylose acetates[10] indicated that the solute was associated to some extent in pyridine, was less associated in pentanedione and methyl acetate and was least associated in chloroform. Even so, it was found that amylopectin acetate prepared by the method of Pacsu,[15] using aqueous pyridine to disperse the amylopectin, and anhydrous pyridine as the acetylation medium, gave a product which was largely insoluble even in chloroform. The product swelled, but only a minor amount appeared to dissolve. A remarkably different amylopectin acetate results when acetylation is performed using formamide as the dispersing agent. Both corn and tapioca amylopectin acetates prepared by this method are readily and completely soluble, without swelling, in chloroform from which results it was immediately apparent that the acetates prepared using aqueous pyridine, followed by extensive heating to dehydrate the reaction medium and the carbohydrate, were very much more highly associated than the products used in the present study. The production of these highly soluble forms of amylopectin acetates has made possible the measurement of osmotic pressures over a wide range of concentration in chloroform, from 0.1 to 2.0 g. per 100 ml., and has facilitated a determination of the slope and shape of the osmotic pressure concentration curves with more certainty than was formerly possible. In experiments on high molecular weight amylopectin samples it has now been observed that the pressure-concentration curve changes slope to a negative value as infinite dilution is approached, and that the limiting pressure at infinite dilution is very much higher than would have been calculated from extrapolation of curves plotted over narrow concentration ranges, *e.g.*, 0.3 to 0.9 g. per 100 ml., such as were used in our earlier studies. It appears, furthermore, that limiting pressures for corn and tapioca amylopectin acetates as shown in Table I (see also Fig. 1), correspond to DP_n values of 1450 and 1300, respectively. Potato amylopectin acetate prepared by the formamide method was, however, insoluble in all solvents tried, including chloroform, and only swelled to form a gel-like mass. Possibly formamide is not a sufficiently powerful dispersing agent for a highly associated potato amylopectin, or possibly the molecules in potato amylopectin may be covalently cross-bonded by phosphate groups, known to exist in the native amylopectin and which account for its very high viscosity.[16]

Confirmation that the DP_n values for corn and tapioca amylopectins are of the order of magnitude as given above has been obtained by several lines of study. The enzymolysis of corn amylopectin and a determination of the osmotic pressures of the limit dextrin acetate has been reported recently by Kerr and Cleveland.[9] This relatively smaller molecule should associate less than any other starch product because of its structure. From a DP_n value of 800, for the limit dextrin acetate prepared by the formamide dispersion method,

TABLE I

OSMOTIC PRESSURES OF THE ACETATES OF B-FRACTIONS AND STARCH IN CHLOROFORM AT 30°

C, concn., g. per 100 ml.	P, mm. of chloroform	π, g. per cm.^{2}	π/C
Corn B-fraction, calcd. DP_n 1440			
0	(By extrapolation)		0.62
0.0995	0.35	0.052	.52
.1011	.35	.052	.52
.2001	.55	.081	.41
.3066	.60	.088	.29
.4218	.90	.132	.31
.6209	1.10	.162	.26
.8608	1.90	.280	.33
1.1046	2.30	.339	.31
1.6008	6.00	.884	.55
1.7735	6.40	.942	.53
2.027	8.60	1.267	.63
Tapioca B-fraction, calcd. DP_n 1275			
0	(By extrapolation)		0.70
0.1054	0.45	0.066	.63
.1032	.40	.059	.57
.2095	.70	.103	.49
.3047	.95	.140	.46
.3250	.95	.140	.43
.4171	.90	.133	.32
.4560	.95	.140	.31
.6181	1.20	.177	.29
.8049	1.95	.287	.36
1.0020	3.95	.582	.58
1.4392	8.90	1.310	.91
Corn starch, calcd. DP_n 1000			
0	(By extrapolation)		0.90
0.1128	0.60	0.0883	.78
.1136	0.60	.0883	.78
.2173	1.10	.162	.75
.3164	1.60	.236	.74
.4081	2.10	.309	.76
.6051	3.10	.456	.75
.7991	4.20	.618	.77
1.2218	8.65	1.274	1.04
1.6144	13.90	2.046	1.27

(15) J. W. Mullen and E. Pacsu, *Ind. Eng. Chem.*, 34, 1209 (1942).

(16) M. Samec, "Kolloidchemie der Stärke," Dresden, 1927.

and from the dextrin yield of 46%, a calculated DP_n value of 1800 is obtained for the parent amylopectin.

TABLE II

OSMOTIC PRESSURES OF THE ACETATES OF B-FRACTIONS OF ACID-HYDROLYZED CORN STARCH

C, concn., g. per 100 ml.	P, mm. of chloroform	π g. per cm.^{2}	π/C
	10-F, calcd. DP_n 920		
0	(By extrapolation)		0.97
0.1015	0.60	0.088	.87
.1006	.55	.081	.81
.2016	.70	.103	.52
.3024	1.00	.147	.49
.5006	1.60	.236	.47
.9986	3.70	.545	.55
1.5080	6.30	.927	.62
	20-F, calcd. DP_n 625		
0	(By extrapolation)		1.35
0.1070	0.90	0.132	1.24
.2000	1.50	.221	1.10
.3199	1.90	.280	0.88
.4291	2.35	.346	.81
.6023	3.10	.455	.76
.8098	4.50	.663	.82
1.0058	5.40	.795	.79
1.5492	10.10	1.490	.96
	40-F, calcd. DP_n 565		
0	(By extrapolation)		1.58
0.1028	0.95	0.140	1.36
.2015	1.35	.199	0.99
.3085	1.65	.243	.79
.4529	2.35	.346	.76
.6047	3.50	.515	.85
.8006	4.75	.699	.87
1.2134	8.15	1.200	.99
	60-F, calcd. DP_n 525		
0	(By extrapolation)		1.70
0.0997	1.0	0.147	1.48
.2034	1.9	.280	1.38
.3084	2.8	.412	1.34
.4486	3.45	.508	1.13
.5996	4.1	.604	1.01
.9972	6.8	1.00	1.00
1.3940	12.0	1.77	1.27
	80-F, calcd. DP_n 260		
0	(By extrapolation)		3.45
0.0503	1.05	0.154	3.08
.1010	1.90	.280	2.77
.2068	3.73	.550	2.66
.3132	5.85	.861	2.75
.4973	10.10	1.487	2.99
.5207	10.70	1.575	3.02
.7259	15.90	2.340	3.22
1.0210	24.80	3.650	3.58
	90-F, calcd. DP_n 210		
0	(By extrapolation)		4.25
0.1015	2.9	0.427	4.21
.2988	8.4	1.240	4.14
.4466	12.6	1.856	4.16
.6089	18.0	2.651	4.35
.7940	26.2	3.858	4.86

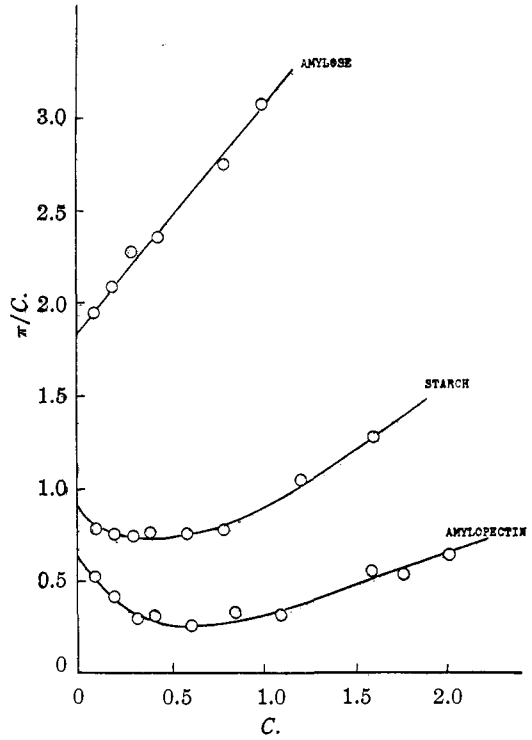

Fig. 1.—Osmotic pressure relationships at various concentrations (C) in g. per 100 ml. of chloroform for triacetates of corn starch, amylose and amylopectin.

The DP_n of corn amylopectin was investigated further by examination of the parent starch of which it is a component. Defatted corn starch was converted into its triacetate after dispersion in formamide. Osmotic pressure values in chloroform are given in Table I and Fig. 1, from which a DP_n of 1000 is calculated. Using a DP_n value of 500 for the amylose component of corn starch[10] which constitutes substantially one-quarter of the weight of the whole starch, a DP_n value (x) of 1500 for the amylopectin component is obtained from the equation

$$\frac{25}{500} + \frac{75}{x} = \frac{100}{1000}$$

Considerable significance is attached to this experiment. In the first place, the osmotic pressures of whole corn starch acetate in chloroform are of such magnitude that they are reproducible with satisfactory precision even at low concentrations, using our technique. It seems improbable that the order of magnitude is otherwise than indicated from values plotted in Fig. 1, unless the acetate molecules still are significantly associated in the solutions employed in which case our values for DP_n would be higher than the true ones. Furthermore, in using unfractionated starch, the possibility of unintentional degradation of starch components during fractionation and purification procedures, as well as a possible loss of significant quantities of subfractions of very low or high DP_n during purification procedures have been eliminated. Again it would appear that maximal DP_n values are obtained for amylopectin by this experiment and we may discard the periodically

entertained view that the molecular weight of amylopectin is measured in millions.

Inasmuch as there is some reason for believing that the tendency of starch molecules of similar structure to associate is a function of molecular size, a study was made of a series of acid-hydrolyzed amylopectin samples. Osmotic pressures were correlated not only against other physical data for members of this series, but with chemical data as well. It was anticipated that relationships found when lower members of the series were studied, the DP_n of which is determined by osmometry with considerable confidence, could be extended to the parent amylopectin and thus by independent methods confirm the DP_n value found for this material. The starches, from which this series of acid-hydrolyzed B-fractions were obtained are similar to the "thin boiling" starches of commerce and the fractions are listed in the tables according to an arbitrary fluidity scale used in industrial practice. Osmotic pressure data for their acetates in chloroform are given in Table II and representative curves are shown in Fig. 2.

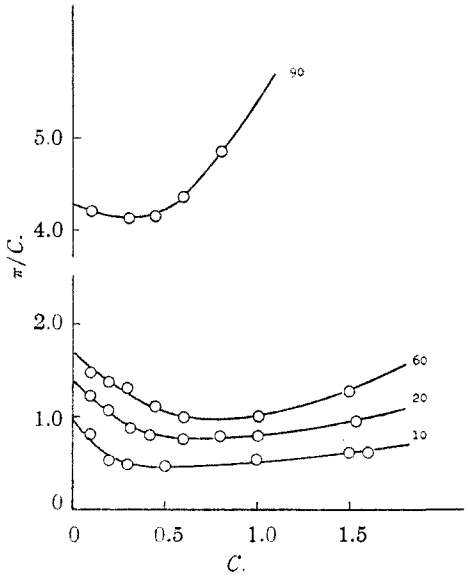

Fig. 2.—Osmotic pressure relationships for the B-fraction acetates of several acid hydrolyzed corn starches between 10 and 90 fluidity.

Several other B-fraction materials of various DP_n values, available in our laboratories, were also included in this study: a B-fraction obtained by hydrolysis of tapioca starch with a *B. subtilis*–α-amylase preparation at temperatures above the gelatinization point, a sample of commercial "glycogen" of comparatively low *DP* and three corn amylopectin sub-fractions prepared according to the method outlined by Kerr.[17] Alkali numbers were determined on all of these B-fractions. The results were compared with DP_n values in Table III and the results plotted in Fig. 3. A hyperbolic function was obtained from which the equation was derived.

$$DP_n = \frac{6700}{\text{alk. no.}} - 30$$

(17) R. W. Kerr, *Arch. Biochem.*, **7**, 377 (1945).

Calculations of DP_n from alkali numbers are shown in Table IV. It is apparent that the equation holds satisfactorily over wide limits of DP_n,[18] with two exceptions, and it also appears that the calculated value for corn amylopectin agrees very well with that obtained directly by osmotic pressure measurements.

TABLE III

CHARACTERISTICS OF SOME BRANCHED STARCH FRACTIONS AND "GLYCOGEN"

Fraction	DP_n, (osmotic pressure)	% light trans. dinitrosalicylate reacn.	Alkali number	$[\eta]$ N KOH at 35°	Ferricyanide number
Potato B-fraction	..	80.8	3.53	1.58	0.25
Corn B-fraction	1450	73.5	4.81	1.25	0.46
Tapioca B-fraction	1300	73.8	4.37	1.35	0.25
Corn amylopectin					
Sub-fraction I	1800	76.8	3.20	1.45	0.38
Sub-fraction II	1200	67.0	5.64	1.06	0.54
Sub-fraction III	450	22.0	13.7	0.53	
Corn limit dextrin	800	52.8	5.30	1.17	0.79
B-fractions, corn					
10 Fluidity	920	53.9	7.05	1.07	0.59
20 Fluidity	625	39.4	9.74	0.70	0.85
40 Fluidity	565	35.5	10.8	0.65	0.91
60 Fluidity	525	31.5	11.1	0.58	1.00
80 Fluidity	260	..	25.9	0.26	3.31
90 Fluidity	210	5.5	27.6	0.295	4.27
B-fraction, tapioca α-amylase conv.	420	20.9	13.50	0.55	1.46
"Glycogen"[a]	500	28.0	8.50	0.069	1.13

[a] Commercial sample, Nutritive Biochemicals Corp.

TABLE IV

CALCULATION OF DP_n FROM ALKALI LABILITY AND FERRICYANIDE OXIDATION VALUES

Substance	DP_n from alkali no.	DP_n from ferricyanide no.	DP_n found by osmotic pressure
Corn B-fraction	1365	1300	1450
Tapioca B-fraction	1505	2400	1300
Corn amylopectin			
Sub-fraction I	2065	1600	1800
Sub-fraction II	1160	1100	1200
Sub-fraction III	465		450
Corn limit dextrin	1240	770	800
Corn B-fractions			
10-F	920	1020	920
20-F	660	700	625
40-F	590	660	565
60-F	570	600	525
80-F	230	185	260
90-F	215	140	210
B-fraction, tapioca α-amylase conversion	470	410	420
"Glycogen"[a]	760	530	500

[a] Commercial sample, Nutritive Biochemicals Corp.

(18) In the alkali lability test, a definite time limit is provided during which a certain number of glucose groups, probably on each starch molecule, are degraded to acidic bodies. Accordingly, the equation could not be expected to hold for starch samples of DP_n so low that the molecules (of even some of them) did not contain at least the number of glucose units that can be degraded during the time interval of the reaction.

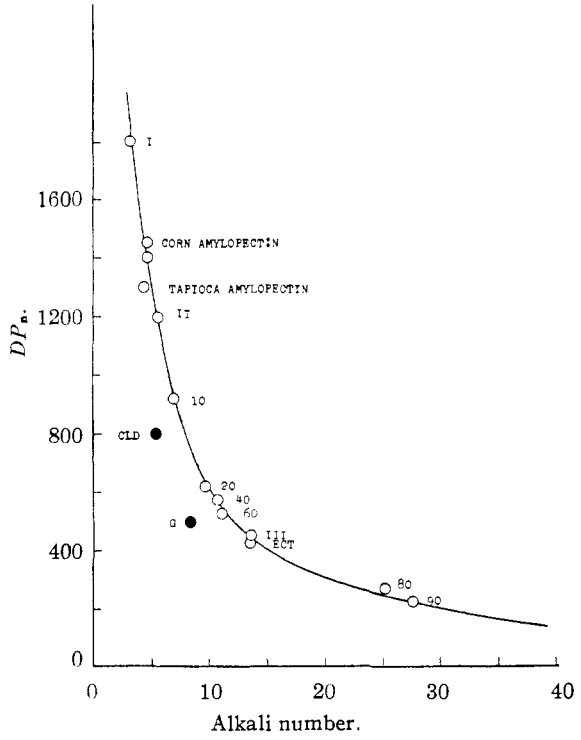

Fig. 3.—Alkali numbers of branched starch materials plotted against DP_n: numbers I, II, III are sub-fractions of corn amylopectin; CLD, corn limit dextrin; ECT, enzyme converted tapioca B-fraction; G, commercial sample of glycogen (see text).

Two exceptions of the DP_n–alkali number relationship for branched fractions are the commercial sample of "glycogen" and corn limit dextrin. It has been recognized by us previously that the alkali number of a B-fraction is less than the alkali number of an A-fraction of substantially the same DP_n. Thus, corn amylose (A-fraction) has an alkali number of 22 whereas the enzyme converted tapioca B-fraction of the same order of DP_n has an alkali number of 13.5; corn crystalline amylose of DP_n 225 has an alkali number of 35 whereas the 90-F B-fraction of equal DP_n has an alkali number of 27.6. It now appears further that the alkali number of a starch material is generally reduced as the percentage of branched points in the structure increases.

Further confirmation of the osmotically determined DP_n value for corn amylopectin was obtained by oxidation of B-fractions listed in Table III with alkaline ferricyanide. The DP_n values are plotted against ferricyanide numbers in Fig. 4. Again a hyperbolic function was obtained from which the following equation was derived.

$$DP_n = 600/\text{F. no.}$$

Calculations made by use of this equation are shown in Table IV. The DP_n calculated for corn amylopectin from its ferricyanide number is of the same order as is obtained by osmotic pressure measurements.

The very low ferricyanide number found for tapioca amylopectin has no obvious explanation, although it may result from certain bleaching treatments commonly employed in the industrial manufacture of root and tuber starches.

The several B-fractions listed in Table III were treated with alkaline dinitrosalicylate and resulting color values determined according to a modification of the method used by Meyer and co-workers.[1] These results are plotted in Fig. 5 as per cent. light transmission values at 500 mμ (after correcting for turbidity) against DP_n values, since it had previously been observed that starch A-fractions gave a substantially linear relationship, as is also indicated in Fig. 5. Although the B-fractions of lower DP_n gave results approaching this linear graph, B-fractions of higher DP_n showed a departure as indicated in the figure. Either these large B-fractions break down more than corresponding linear fractions during the dinitrosalicylate treatment, to give more color (a possibility not indicated from the results with branched fractions of low DP_n) or, some association still exists in the large B-fraction acetates, even when prepared by improved procedures, and their true DP_n values are somewhat less than determined by osmotic pressure measurements.

The intrinsic viscosities of the B-fraction series are compared against DP_n values in Table III and Fig. 6. A slightly curvilinear relationship results if the curve is extended through the origin. Some deviations from the curve are to be expected in view of the various degrees of polydispersibility in the many fractions tested. The very colloidally stable and polydisperse tapioca amylopectin would be expected to have a higher viscosity than the less polydisperse subfractions of corn amylopectin. However, the viscosity relationship does assist in confirming the DP_n value for amylopectin by osmotic pressure studies, particularly when taken with other confirmatory results presented.

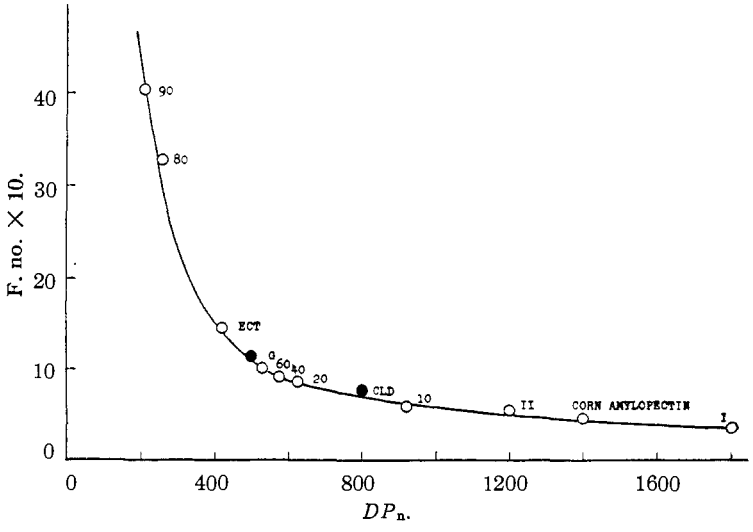

Fig. 4.—Ferricyanide numbers of branched starch materials plotted against DP_n.

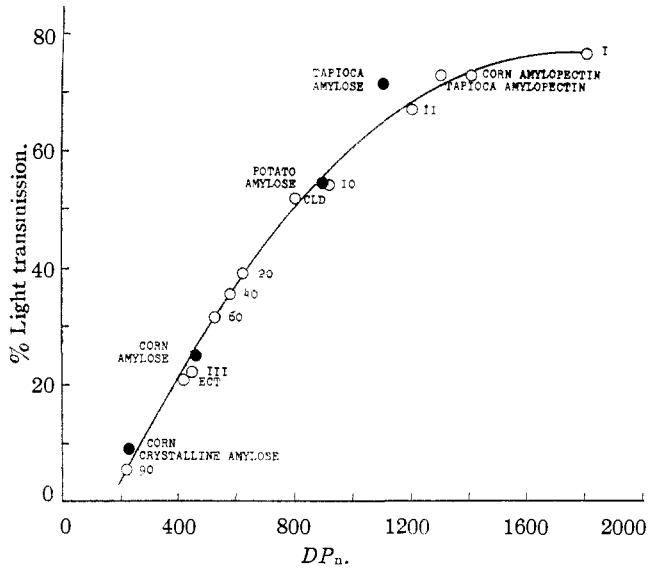

Fig. 5.—Per cent. light transmission of colors produced by dinitrosalicylate with branched starch materials plotted against DP_n. Solid circles are for linear starch fractions.

Two exceptions to the viscosity–DP_n relationship are "glycogen" and corn limit dextrin. The "glycogen" sample has a very low viscosity, indicative of a ramified, fairly symmetrical shape. The limit dextrin has an exceptionally high viscosity and therefore probably is quite different in structure from glycogen. Moreover, since the intrinsic viscosity of the limit dextrin is almost as high as the parent amylopectin, it may be that the enzymic hydrolysis leading to formation of the dextrin decreased the length of the parent amylopectin molecule in about the same proportion as it did the side branches.

Osmotic pressure measurements and dinitrosalicylate color values indicate that tapioca amylopectin is of slightly lower DP_n than corn amylopectin, whereas alkali number and intrinsic viscosity indicate that the DP_n of tapioca amylopectin is higher. The order of DP_n is possibly 1300–1400.

We have not succeeded in producing a solvent-soluble potato amylopectin acetate. Dinitrosalicylate color value of 80.8% light transmission, an alkali number of 3.53 and an intrinsic viscosity of 1.58 indicate a DP_n of 2000, or higher.

According to Steurer[19] osmotic pressure curves for ethyl cellulose in benzene and toluene which show minima at low solute concentrations indicate association effects which become less as infinite dilution is approached.[20] Association between solute molecules would naturally be expected to take place more with the linear amylose than with the branched amylopectin. Previously,[10] we were unable to find osmotic pressure minima for amylose acetates prepared by Pacsu's aqueous pyridine dispersion method. The osmotic pressures for corn amylose acetate were redetermined, using a triacetate prepared by the formamide dispersion method and using a wider range of concentrations in chloroform. The results are given in Table V and are plotted in Fig. 1. The amylose preparation No. 27 gave a curve almost superimposable on the one previously reported and a calculated DP_n value of 480, which is very nearly within the experimental error of the value, 455, obtained in earlier work. Potato amylose (Pentasol precipitated, butanol recrystallized) was likewise found to give the same DP_n of 850 and a linear osmotic pressure curve whether the acetate was prepared with acetic anhydride after dispersion in aqueous pyridine or in formamide. It is not readily apparent why amylose acetate should give the more commonly observed linear osmotic pressure curve whereas amylopectin acetate does not. It follows, however that the unusual shape of the amylopectin acetate curve is not due to some obscure action of formamide, *per se*, and it would appear that the type of association between amylopectin acetate molecules, if it exists, is different from that usually observed between amylose molecules.

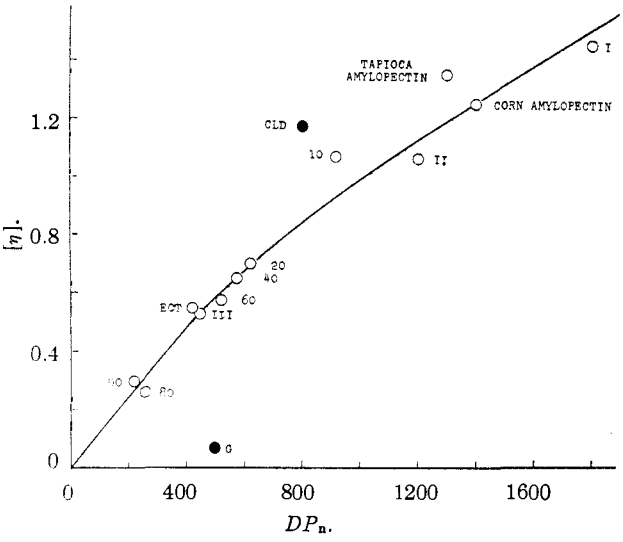

Fig. 6.—Intrinsic viscosity in N KOH at 35° for branched starch materials, plotted against DP_n.

TABLE V

OSMOTIC PRESSURES OF CORN AMYLOSE ACETATE PREPARED BY THE FORMAMIDE DISPERSION METHOD

C, concn., g. per 100 ml.	P, mm. of chloroform	π, g. per sq. cm.	π/C	Calcd. DP_n
0	(By extrapolation)		1.85	480
0.1057	1.4	0.206	1.95	
.1977	2.8	.411	2.08	
.3039	4.7	.691	2.27	
.4448	7.1	1.046	2.35	
.6068	9.8	1.440	2.38	
.7989	14.9	2.190	2.75	
1.0140	21.1	3.120	3.08	

(19) M. E. Steurer, *Z. physik. Chem.*, **A190**, 1 (1941).

(20) Spurlin and co-workers (see H. M. Spurlin in "Cellulose and Cellulose Derivatives," by E. Ott. Interscience Publishers, New York, N. Y., 1943) and particularly H. M. Spurlin, and E. F. Evans, THIS JOURNAL, **72**, 4750 (1950)) have studied these anomalous effects in some detail and have proposed that they are the result of bound metals. It is possible that the effects here reported for amylopectin acetate in chloroform are due to ionic groups, perhaps bound metals on phosphate ester groups. Experiments are in progress in our laboratories to test the proposal of Dr. Spurlin with amylopectin.

Native glycogen is usually conceded to have a very high molecular weight of a million or more and some of the current estimates of the molecular weight of the amylopectins have given a value of this order also. Inasmuch as our work does not support this conclusion for amylopectin, it became of interest to examine native glycogen under our experimental conditions. A sample of freshly prepared dog liver glycogen was obtained from Dr. G. T. Cori, isolated according to the method of Somogyi. After acetylation by the formamide dispersion method, osmotic pressures in chloroform were obtained as shown in Table VI. It is apparent

TABLE VI

OSMOTIC PRESSURES OF THE ACETATE OF DOG LIVER GLYCOGEN

C, concn., g. per 100 ml.	P, mm. of chloroform	π, g. per cm.^{2}	π/C	Calcd. DP_n
0	(By extrapolation)		0.17	5300
0.0954	0.10	0.0150	.154	
.1946	.25	.0368	.189	
.2857	.35	.0515	.179	
.4276	.55	.0809	.189	
.5785	.70	.1029	.178	
.7947	.95	.1397	.158	
1.3032	1.35	.1985	.152	

that the function of π/C is very constant and a limiting value of 0.17 was readily estimated. This is equivalent to a DP_n of 5000–6000. After this experiment was completed, the osmometer cell was thoroughly washed with chloroform and the π value for glycogen at a concentration 0.1 g. per 100 ml. was checked at 0.015 g. per sq. cm. After a second washing, corn amylopectin acetate at the same concentration was introduced and a π value of 0.059 was obtained. Thus, further confirmation was provided that at very low concentrations, the osmotic pressure of the amylopectin acetate is almost four times as great as the glycogen acetate.

As a concluding experiment to demonstrate the possible influence of a "constant error" in either equipment or procedure in producing the anomalous osmotic pressure results at very low concentrations, the experiment with corn amylopectin acetate was repeated with the following significant changes. Instead of using a new membrane, a cell set-up was used which had been previously employed for a period of three weeks with another starch fraction acetate. The cell was washed with chloroform and instead of starting with very low concentrations and increasing this value, the relatively high concentration of 0.45 g. per 100 ml. was used first followed by progressively more dilute solutions. These results, shown in Table VII, confirm the results in Table I of the first given experiment with amylopectin acetate, which demonstrates a negative slope for the osmotic pressure curve as infinite dilution is approached.

TABLE VII

OSMOTIC PRESSURES OF CORN AMYLOPECTIN ACETATE USING PROGRESSIVELY MORE DILUTE SOLUTIONS

C, concn., g. per 100 ml.	P, mm. of chloroform	π, g. per sq. cm.	π/C
0.4472	0.80	0.118	0.26
.2900	.65	.096	.33
.1977	.55	.081	.41
.0996	.35	.0515	.52
.0989	.40	.059	.59
0	(By extrapolation)		.65

Summary

Osmotic pressures of corn amylopectin acetate in chloroform indicate a DP_n of the order of 1400–1500, compared to a value 1800 previously estimated by means of enzymic hydrolysis. Tapioca amylopectin was found to have a DP_n of 1300–1400 and a chloroform soluble potato amylopectin acetate could not be prepared.

Whole corn starch was found to have a DP_n of 1000 by osmometry consistent with the view that it is composed of one part amylose of DP_n 500 and 3 parts amylopectin of DP_n 1500.

Additional confirmation for the osmotically determined DP_n values for the amylopectins was obtained from the relationships determined between DP_n and viscosity, alkali lability, ferricyanide number and dinitrosalicylate color values for acid-hydrolyzed corn B-fractions, enzyme-hydrolyzed B-fraction, and corn amylopectin subfractions, followed by extrapolation of these relationships into higher orders of DP_n.

A sample of commercial "glycogen" (DP_n of 500) showed an abnormally low viscosity and alkali number. Corn limit dextrin also was found to have a low alkali number but an abnormally high viscosity, almost as high as corn amylopectin from which it was prepared, indicative of a molecular shape quite different from a highly ramified glycogen molecule. Dog liver glycogen was found to have a DP_n of 5000–6000 and an extremely low viscosity.

Osmotic pressures of corn amylose acetate, prepared by the formamide dispersion method, which was used for the preparation of amylopectin acetates in this study, agreed with values previously reported using the aqueous pyridine dispersion method for derivatization.

ARGO, ILL. RECEIVED APRIL 10, 1950