[CONTRIBUTION FROM THE STARCH AND DEXTROSE SECTION, NORTHERN UTILIZATION RESEARCH BRANCH[1a]]

Characterization and Classification of Dextrans from Ninety-six Strains of Bacteria[1b]

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Heretofore relatively few of the dextrans potentially available through bacterial fermentation have been prepared and characterized. The rapidly increasing signifiance of these polyglucosans for medical, industrial and research purposes motivated a survey of the types obtainable. We have prepared dextrans of high purity from 96 individual bacterial strains and characterized them by periodate oxidation–reaction analysis, by measurement of optical rotation, intrinsic viscosity, the concentration-dependent parameter of viscosity and infrared absorption, and by observations on solubility and gum properties. All the dextrans contained 1,6- or 1-glucosidically linked units, in percentages of 50–97, as well as 1,4-like and/or 1,3-like linked units; the lowest percentages of 1,4-like and 1,3-like linked units were within the limits of error of the periodate method, the highest percentages were about 50 and 40, respectively. On the basis of the proportions of 1,3-like links as indicated by periodate oxidation–reaction analysis, these dextrans have been grouped into 3 classes which contain (A) 0–2%, (B) 3–6% and (C) >6% 1,3-like links. Most strains yielded dextrans that could be placed in one or another of these classes; 6 strains, however, elaborated structurally heterogeneous dextrans the components of which belong to different classes. Our dextrans might not yet represent all possible classes, and reclassification of some of them might be indicated when more specific structural analyses become available.

The particular dextrans which were used initially in this country and abroad for conversion into synthetic blood-volume expanders came into their role more through force of circumstances than through known superiority for the purpose. When the clinical use of dextran was initiated in Sweden in 1944[2] and in other countries more recently, only a small number of dextrans had been reported in the chemical literature,[3–14] and not all of these had been well characterized chemically. It was quite certain, however, that these dextrans were homologous polymers of glucose with predominantly α-1,6-links. Differences had been found among dextrans from several strains of *Leuconostoc mesenteroides*[4,10,13] and from related organisms.[6,7,13] However, there was uncertainty as to whether the differences were dependent upon the microörganism or were due to the conditions for culturing the organisms,[3a] to the methods for isolating the dextrans,[14,15] or to the degree of purity of the dextrans. The situation was clarified by a demonstration of the biological origin of pronounced differences in the structure of dextrans from 4 strains of *L. mesenteroides*.[16]

These evidences of the variation among dextrans, together with the knowledge that dextran-producing microörganisms were widely distributed and rather diverse in growth characteristics,[17,18] indicated the need for systematic chemical and physical study to determine the range of diversity of the polysaccharides from many different microörganisms of this type. Furthermore, data were needed which would make possible the selection of dextrans most suitable for specific applications in medicine, in fundamental research and in industry.

Such a survey was initiated, and reported here are our results on the isolation, purification, characterization and classification as to structural type of dextrans from 96 strains of bacteria. All except 2 of these strains produced their dextrans from sucrose; 2 *Acetobacter* strains transformed amylaceous dextrin into dextran.[19]

These dextrans have been characterized through determination of the chemical nature and proportions of glucosidic linkages present by periodate oxidation,[20] through measurement of specific rotation, viscosity and infrared absorption, and through observations on the solubility and the physical appearance of the highly hydrated gums and of their aqueous solutions. On representative types of these dextrans, structural determination by methylation analysis is being carried out,[21] as well as particle weight and other physical measurements[22] and serological studies.[23] Microbiological aspects of this survey will be reported elsewhere.[24]

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(1b) Presented at the Symposium on Microbial Polysaccharides at the 122nd national meeting of the American Chemical Society, Atlantic City, N. J., Sept., 1952.

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(3) Space restrictions prevent citation of all possible references. Extensive compilations of references on dextran are as follows: (a) T. H. Evans and H. Hibbert, "Advances in Carbohydrate Chemistry," Vol. 2, Academic Press, Inc., New York, N. Y., 1946, p. 203; (b) Allene Jeanes, "Dextran—A Selected Bibliography," AIC 288 Revised, Northern Reg. Res. Lab., Peoria, Ill., 1952, 42 pp.

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TABLE I

PROPERTIES AND CLASSIFICATION OF PURIFIED DEXTRANS FROM 96 DIFFERENT STRAINS OF BACTERIA. IDENTITY AND ORIGIN OF THE STRAINS

Strain no. NRRL B-	Dextran: Type AGU links, % 1,6-	1,4-like	1,3-like	Dextran: $[\alpha]^{25}D$ (c 1) HCONH$_2$	1 N KOH	Dextran: Viscosity, water, 25° $[\eta]$	k_1	Yield, %[a]	Solubility, water[b]	Nature of product[c]	Strain: Identity[d]	Donor and donor's no.[e,f]		Other strain no. and ref.
Class A Dextrans. 0-2% 1,3-like links														
1146	97	3	0	+214		1.245	1.07	11	+	Long	L.d.	NCIB	3356	P-2615[17]
1064	96	4	0	214		0.887	0.91	29	+ p	Tough, stringy	L.m.	CSMc	548	Type D[18]
1414	96	4	0	214		.869	.96	15	+	Short	L.m.	Isolate		
1145	96	2	2	214		1.029	.83	24	+	Long	L.d.	NCIB	3355	52[17]
512(F)[27]	95	5	0	215	+203	0.953	1.10	24	+	Long	L.m.	RGB[14]		Substrain of B-512[27]
640	95	5	0	214		1.280	1.03	14	+	Long	L.d.	ATCC	8086	22[17]; its dextran[7]
1066	95	5	0	215		0.521	0.83	11	+ p	Crumbly, F[9]	L.m.	CSMc		Subtype of type D[18]
1208	95	5	0	213		.628	1.37	17	+ p	Crumbly	L.m.	CSMc		Type D[18]
1210	95	5	0			.698	1.33	24	+	Short, rough	L.m.	CSMc		Type D[18]
1211	95	5	0	214		.843	1.30	16	+	Short, smooth	L.m.	CSMc		Type D[18]
1308	95	5	0	219		.476	1.37	15	+ p	Pasty, crumbly, F	L.m.	EJH		
1209	95	3	2	215		.693	0.87	18	+	Short, smooth	L.m.	CSMc		Type D[18]
1119	94	4	2	217		1.617	.86	7	+	Cohesive, stringy	L.m.	ATCC	8357	
1072	94	6	0	216		0.883	.93	24	+	Long	L.m.	ARS		Substrain of B-512
1198	94	6	0	215		.760	1.39	23	+	Short, F	L.m.	CSMc		Type D[18]
1212	94	6	0					16	+	Short	L.m.	CSMc		Type D[18]
1380	94	6	0	215		.848	1.39	4	+	Short	u	ALP		Similar to strain reported[28]
-I	93	7	0			.968	1.17	2	+	Short, tough				
1405	94	6	0	216		.660	1.34	9	+	Short	L.m.	Isolate		
1412	94	6	0	216		1.127	0.98	12	+	Long	L.m.	Isolate		
1413	94	6	0			0.704	1.21	15	+	Short	L.m.	Isolate		
1417	94	6	0	217		.654	1.14	17	+	Short, F	L.m.	Isolate		
1442	94	6	0	214		1.019	0.86	16	+	Fluid, stringy	L.m.	CSMc		Type A[18]
1204	93	7	0			0.846	1.28	18	+	Crumbly	L.m.	CSMc		Type D[18]
1214	93	7	0					21	+	Short, F	L.m.	CSMc		Type D[18]
1197	92	6	2	212		.510	1.13	8	+	Floc. ppt.	L.m.	CSP	683	9[17]
1307	91	9	0	215		.952	1.08	19	+ p	Short, tough	L.m.	JMN, EJH	"B"	References 10, 29, 30
1388	91	9	0			.917	1.09	16	+	Short, tough	L.m.	RP		
1225	90	10	0	208				24	+ p	Short	A.c.	EJH		NCTC 4943, Ref. (19)
1226	90	10	0	212		.704	0.87	20	+ p	Short	A.v.	EJH		NCTC 7216, Ref. (19)
1500	90	10	0	215	204	.823	1.71	19	+ p	Short, tough	(L.m.)	CSMc		Type F[18]
1415	89	11	0	216		1.180	0.91	12	+	Stringy	L.m.	Isolate		
1196	88	10	2	215		0.890	1.22	26	+	Short	L.m.	WWC	"elai"	Ref. (31)
1409	86	14	0			.950	1.02	7	+	Stringy	L.m.	Isolate		
1383	84	16	0	217		.957	1.12	15	+ p	Short, rough	L.m.	RP		
1416	84	16	0	216		.875	0.89	17	+	Short	L.m.	Isolate		
1525	83	17	0	217		.843	0.88	24	+	Fluid, stringy	L.m.	Isolate		
1390	82	18	0	216		.857	1.03	14	+ p	Short, stiff	L.m.	RP		
1382	81	19	0	218		.840	1.09	13	+ p	Short	L.m.	RP		

1396	81	19	0	217			19	+	Short, tough	L.m.	Isolate		
1420	81	19	0	216		.522	0.68	8	+	Short	L.d.	Isolate		
-I	80	20	0	214		.453	1.23	7	+	Short				
1526	79	21	0	216		.378	0.65	4	+		S.sp.	Isolate		
-I	77	23	0			.225	1.42	8	+	Short				
1397	75	25	0	219				21	+	Short	L.m.	Isolate		
1422	74	26	0	218		1.027	0.89	19	+	Short	L.m.	ERW		
1424	72	28	0	219		1.088	.75	17	+	Stringy	(L.m.)	JW	"D"	Refined Syrups and Sugars, Inc., strain "D" or 1053; derived from ATCC 6025
1402	66	34	0	220		0.925	.78	21	+	Short, F	L.m.	ERW		
1399	65	35	0	217		.913	.84	19	+	Short	L.m.	Isolate		
1298	64	36	0	223		1.025	.90	12	+ p	Short	L.m.	JMN	7 or "C"	Serol. type A[29,30]
									Class B Dextrans. 3–6% 1,3-like links					
1193	95	2	3	+218		0.578	1.34	5	+	Short	L.d.	CSP	853	
641	94	3	3	215		1.041	1.02	17	+	Long	L.m.	ATCC	8082	
1205	94	3	3			0.865	1.22	26	+	Short, F		CSMc		Type D[18]
1387	94	3	3	217		1.418	1.04	14	+	Short	L.m.	RP		
1407	94	3	3	216		0.572	1.73	13	+	Short, cohesive	L.m.	Isolate		
1419	94	3	3	217		.815	1.45	19	+	Short, tough	L.m.	Isolate		
1400	93	3	4	220		.795	0.87	5	+	Short	L.m.	Isolate		
1401	93	3	4	215		.446	.87	8	+	Short	L.m.	Isolate		
1394	92	4	4	215		2.020	.70	6	+	Cohesive, stringy	L.m.	FWF[32]		
-I	92	5	3			1.472	1.44	1	+					
1410	91	5	4	217				7	+	Short	L.m.	Isolate		
1392	91	6	3	218		0.555	0.96	9	+	Stringy	u	Isolate		
1255	89	7	4	219		.696	1.22	18	+ p	Floc. ppt., crumbly	S.d.	AJK	L-337	Isolation[33]
1127	89	5	6	220		.945	0.87	14	+ p	Long	B.v.	AJK	L-343	May be same as previously reptd.[6]
1502	87	8	5		208	1.043	.97	7	+ p	Short	(L.m.)	CSMc		Type F[18]
1144	87	7	6		209	1.153	.72	9	+ 120°	Short, tough	L.m.	NCIB	3354	
									Class C Dextrans. >6% 1,3-like links					
1120	85	0	15					9	−	Crumbly	L.m.	ATCC	8358	Type I[34]
1351	85	4	11	217		0.505	0.52	27	+	Short	S.v.	EJH		Ref. (35)
1389	85	7	8	220		1.102	1.23	21	+	Short	L.m.	RP		
1429	85	5	10		210	1.360	1.16	8	+ p	Crumbly	L.m.	CSMc		Type B[18]
1377	84	7	9	219		1.364	0.81	20	+	Long	L.m.	Svenska Sockerfabriks	AB VII-E	
1384	84	6	10	221				20	+ p	Tough	L.m.	RP		
1139	83	5	12		213	0.503	1.25	9	+ p 120°	Floc. ppt.	B.v.	AJK	L-344	Isolation[33]
1411	82	8	10	217		1.093	1.07	21	+	Short, tough	L.m.	Isolate		
1385	81	9	10	222	213	0.995	1.22	21	+ p	Crumbly	L.m.	RP		
1374	81	7	12	220		1.338	0.79	25	+	Stringy	L.m.	Benger's Ltd.		
1375	81	6	13	220		0.918	1.00	14	+	Short	L.d.	Dextran Ltd.		"Birmingham" strain[13,36]
1438	81	6	13		213	1.569[k]	0.89	4	+ 120°	Floc. ppt.	L.m.	CSMc		Type B[18]
1438-A	79	7	14		213	1.458[k]	.86	9	+ 120	Floc. ppt.				

TABLE I (*Continued*)

Strain no. NRRL B-	Type AGU links % 1,6-	1,4-like	1,3-like	$[\alpha]^{22}D$ (*c* 1) HCONH$_2$	1 *N* KOH	Dextran Viscosity, water, 25° $[\eta]$	k_1	Yield, %[a]	Solubility water[b]	Nature of product[c]	Strain Identity[d]	Donor and donor's no.[e,f]	Other strain no. and ref.
1439	81	6	13	221		0.475	1.08	10	+	Fluid, stringy	L.m.	CSMc	Type A[18]
1443	80	10	10	220		.418	0.86	18	+	Pasty	(L.m.)	CSMc	Type A[18]
1141	79	3	18	224		1.350	1.04	17	+	Tough, stringy	L.d.	NCIB 2706	63[17]
1192	78	4	18	223	210	0.910	1.33	22	+	Short, crumbly	L.m.	CSP 851	
1191	77	9	14	223		.882	1.35	19	+	Short, crumbly	L.m.	CSP 845	
1118	76	3	21		215	1.821[h]	0.74	9	−	Floc. ppt.	L.m.	ATCC 8293	
1425	74	8	18	222		1.105	.93	7	+	Fluid, stringy	L.m.	CSMc	Type A[18]
1398	70	11	19	222		0.865	.91	19	+	Short	L.m.	Isolate	
1297	67	24	9	219	211			2	+ p, 120°	Short, rough	L.m.	JMN 5 or "A"	Ref. (10, 29, 30)
F90A[37]	67	2	31		225				+ p, 120	Floc. ppt.	S.v.	EJH	Lancefield group H[37]
523	66	10	24		220	2.081[h]	1.51	6	−	Floc. ppt.	L.m.	C. Thom 535	
1121	65	2	33		222			7	−	Floc. ppt.	L.m.	ATCC 8359	Type II[34]
1142	63	8	29	230		0.389	1.60	6	+ p	Floc. ppt.	L.m.	NCIB 3351	Same origin as NRRL B-742
1433	63	30	7		217	2.605[h]	1.17	17	−	Crumbly	L.m.	CSMc	Type B[18]
1433-A	63	30	7		217	2.514[h]	1.22	6	−	Tough			
1431	62	29	9		217	3.107[h]	0.34	10	−	Floc. ppt.	L.m.	CSMc	Type B[18]
1149	52	8	40		232	2.716[h]	1.24	4	−	Fine ppt.	L.m.	NCIB 6109	
						Structurally heterogeneous dextrans and/or their major components							
742	67	21	12	+223		0.296	1.35	15.0	+ p	Short, dense	L.m.	CSP[16] 681	5[17]; 4[4]
-L	81	19	0	212		.152	1.38	(35)[i]	+ p	Fine ppt.			
-S	57	17	26	226		.326	1.45	(39)	+ p	Fine ppt.			
1254	90	7	3	216		.488	1.39	12	+ p	Floc. ppt.	S.d.	AJK L-336	Isolation[33]
-L	69	31	0	213		.189	1.29	(7)	+ p	Floc. ppt.			
-S	93	7	0	214		.537	1.26	(55)	+ p	Floc. ppt.			
1299-L	58	36	6		+216	.873	1.05	(55)	+ p 120°	Floc. ppt.	L.m.	JMN 8 or "K"	From AJK, 1940. Serol. type A[29,30]
-S	50	50	0	221	212	.469	1.53	(23)	+	Fine ppt.			
1355-L	88	9	3		206	1.115	1.13	(37)	−	Short	L.m.	RP	
-S	57	8	35	233	220	0.193	1.24	(48)	+	Fine ppt.			
1498-A	91	9	0	212		1.156	1.81	5	+ p	Short, tough	L.m.	CSMc	Type F[18]
-L	94	6	0	213		1.096	1.30	14	+	Short			
-S	62	11	27	227		0.329	1.25	3	+	Fine ppt.			
1501-A	80	18	2	211		1.004	1.67	7	+ p	Short, dense	L.m.	CSMc	Type F[18]
-L	93	7	0		206	1.054	1.34	7	+	Short			
-S	65	15	20		216	0.412	1.28	5	+	Fine ppt.			

[a] Based on weight of sucrose in culture. [b] +, soluble; −, insoluble; p, if precautions are observed; 120°, solution completed by autoclaving. Observed when precipitated from aqueous solution by ethanol of 45–50% concentration. Products are gums unless otherwise stated. [d] Identities are as confirmed or determined[24] except for those indicated in parentheses, which are as received. A, *Acetobacter;* B.v., *Betabacterium vermiforme;* c, *capsulatum;* d, *dextranicum;* L, *Leuconostoc;* m, *mesenteroides;* S.d., *Streptobacterium dextranicum;* S.sp., *Streptococcus* species; S.v., *Streptococcus viridans;* v, *viscosum;* u, unidentified. [e] NCIB, National Collection of Industrial Bacteria; ATCC, American Type Culture Collection; NCTC, National Collection of Type Cultures. [f] Initials stand for names of donors, as follows: R. G. Benedict; W. W. Carlson; E. J. Hehre; G. J. Hucker; A. J. Kluyver; C. S. McCleskey; J. M. Neill; R. Patrick; C. S. Pederson; A. L. Pollard; A. R. Stanley; J. Warren and E. R. Wolford. [g] I^2, fluorescent in ordinary light as previously reported.[11,34] [h] Solvent, 1 *N* potassium hydroxide. [i] Values in parentheses are per cent. of the purified fraction obtained from the whole dextran.[26]

Experimental

Cultures.—The dextran-producing bacteria employed in this survey were obtained from several culture collections, from other investigators of these microörganisms and of their polysaccharides and by isolation from various natural sources. Each of these pure cultures, which is made up of descendents of a single isolation, is defined as a "strain."[25] Pertinent data are given for each strain in Table I. Although we designate the strains and their respective dextrans by the NRRL number of the strain in the Culture Collection of the Northern Utilization Research Branch, we cite previous designations of cultures as well as publications concerning them or their dextrans.

With a few exceptions, the strains in Table I are believed to be free of duplication; such cultures as originated from a common natural source showed differences in taxonomy or in the physical and chemical characteristics of their dextrans. A few strains which had a common origin but different subsequent history have been retained purposely as separate strains because their dextrans differed significantly (B-742, -1142; B-1424, -1297) or their total fermentation products are of special interest (B-512, -1072).[26]

Dextran Production.—Most of the dextrans reported here were prepared under standardized culture conditions. The set of conditions used could not be expected to be optimal for each organism and doubtless caused yields and viscosities of dextrans from certain strains to be lower than might have been obtained under conditions more adequately fulfilling the specific requirements of those strains. For example, the yield of B-1146 dextran can be increased by inclusion of supplementary vitamins in the medium, and the viscosity of B-512 dextran is decreased sharply when the incubation time is extended, as in this survey, beyond that required for completion of dextran production.[14] However, nearly every organism has been cultured for dextran production under from 2 to 9 different conditions without change in the type of dextran being detectable by our methods.[24] Therefore, reproduction of the dextran preparations reported here can be expected under our conditions of culture propagation and medium composition. It cannot be assumed, though, that the dextran products would remain unchanged under all possible modifications of culture conditions.

The dextrans were isolated from liter cultures after incubation at 25°, usually for 5 days, in a sucrose, tryptone, phosphate medium supplemented with yeast extract, liver extract or malt extract. Essentially anaerobic conditions were encouraged by "deep" culturing. Specific details on the culturing of the organisms, the conditions for dextran production, and the relation of these conditions to the dextran product are to be reported elsewhere.[24]

Special conditions were established for production of dextran from B-1225, -1226 and -1351.[19,38] Dextran from strain F90A, which was the product previously described,[37] was given further purification before being characterized by our methods.

Observations on Cultures.—At the end of the period of dextran production, the cultures presented a variety of appearances. Usually there was no pronounced odor; the *p*H values usually were 4 to 5. The cultures from some strains showed marked fluorescence in ordinary light, as indicated in Table I, and as reported previously by others.[18] Almost invariably the cultures were cloudy or opaque. The gross viscosity of cultures from different strains ranged from almost solid gels and thick fluids to thin fluids, and usually was proportional to the yield of dextran present. Many of the viscous cultures were dull and turbid (notably B-1254, -1255 and -1308); others were glistening and semi-transparent (B-512, -1145, -1146, -640, -641 and -1412). Still other cultures appeared to have a gum phase uniformly dispersed throughout the medium (B-1119, -1394 and -1433). In many cases gum- or gel-like material had separated out on the bottom of the culture flask (notably B-1254, -1255 and -1394, as well as strains requiring extended periods of incubation). Often this second phase appeared to dissolve in the culture when stirred or shaken. In some cases mixing was avoided[26] and this second phase was isolated and purified separately giving the "I" fractions of B-1380, -1394, -1420 and -1526. From cultures that showed flocculent particles suspended in either thin or viscous solutions (B-523, -1118, -1149 and others), water-insoluble dextrans were obtained.

Isolation and Purification of Dextrans. (A). Water-soluble Dextrans.—The cultures were made to 33–35% by volume with ethanol[39]; viscous cultures first were blended with water and diluted to 2 volumes or less with water. These alcoholic solutions were slowly passed twice through a continuous supercentrifuge. This removed bacterial cells, insoluble matter and dextran fractions of low solubility. The concentration of ethanol in the supercentrifugate then was increased just to the point where dextran precipitation appeared complete (usually 42–45% ethanol) or else to 50% ethanol. The supernatant fluid was decanted promptly from gummy products or centrifuged from flocculent precipitates. By kneading or stirring with 50% ethanol and then reprecipitating from water solution 3 successive times by addition of an equal volume of ethanol,[14] the dextran product was purified from adhering nutrients from the medium and from by-products of bacterial fermentation. All of these operations were carried out at room temperature.

For reprecipitations, the dextran products were dispersed most successfully by gradual addition of water and stirring to obtain homogeneous pastes before diluting further. Some products appeared to become less soluble during purification, especially in the presence of 50% ethanol, and required autoclaving at *p*H 5–6 to obtain in about 5% aqueous solution (some B-1254 preparations, B-1431 and -1433). Dextran products readily soluble in water were dehydrated by adding the aqueous solution to absolute ethanol, washing with ethanol and finally drying under anhydrous conditions as previously described.[14] Dextrans less readily soluble (such as B-1139, -1144, -1193, -1299L, -1355L and -1433) were dehydrated in the frozen state under high vacuum (lyophilized) to insure greater ease of dissolution later.

Description will be given elsewhere of the methods used in separating and purifying the polysaccharide fractions insoluble at ethanol concentration of 35% (for example, fractions B-1438-A, -1433-A, -1498-A and -1501-A), as well as those requiring 65% or higher concentrations for precipitation.[26]

(B) **Water-insoluble Dextrans.**—Cultures that showed much insoluble gum or flocculent particles after dilution and vigorous agitation were treated at 25° with 10% potassium hydroxide solution to give a final concentration of 1 *N*. After supercentrifugation, the *p*H was adjusted to 3–4 with acetic acid and the dextran precipitated with ethanol. The product was washed with 50% ethanol and thrice reprecipitated from aqueous solution. Often, potassium hydroxide was necessary for redissolving the dextran in the course of purification (examples: B-523, -1118, -1120 and -1149); such dextrans were given a final precipitation from water suspension to reduce salt content. In other cases, autoclaving the dextran at *p*H 5–6 produced solution (examples: B-1431 and -1433).

(25) R. E. Buchanan, R. St. John-Brooks and R. S. Breed, *J. Bacteriol.*, **55**, 293 (1948).

(26) C. A. Wilham, B. H. Alexander and Allene Jeanes, in preparation for publication.

(27) In 1950, the B-512(F) substrain supplanted B-512 for all work at the Northern Utilization Research Branch. Since that time, the dextran from this substrain has been designated inexactly as B-512 in numerous publications, and will be so designated hereinafter in this paper. The dextrans from B-512 and B-512(F) appear to be identical

(28) P. B. Smith and A. L. Pollard, *J. Bacteriol.*, **63**, 129 (1952).

(29) J. M. Neill, J. Y. Sugg, E. J. Hehre and E. Jaffe, *Proc. Soc. Exptl. Biol. Med.*, **47**, 339 (1941).

(30) E. J. Hehre, *ibid.*, **54**, 18 (1943).

(31) W. W. Carlson and Virginia Whiteside-Carlson, *ibid.*, **71**, 416 (1949).

(32) F. W. Fabian and R. H. Henderson, *Food Research*, **15**, 415 (1950).

(33) L. H. C. Perquin, *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, **6**, 227 (1939–1940).

(34) J. A. Alford and C. S. McCleskey, *Proc. La. Acad. Sci.*, **6**, 36 (1942).

(35) E. J. Hehre, *Bacteriol. Proc.*, 23 (1952).

(36) W. N. Haworth and M. Stacey, Brit. Patent 618,999 (March 2, 1949).

(37) For this dextran, we are indebted to Drs. E. J. Hehre and J. M. Neill, who described its preparation and partial characterization in *J. Exptl. Med.*, **83**, 147 (1946).

(38) E. J. Hehre and H. M. Tsuchiya, unpublished results.

(39) All ethanol concentrations are in terms of absolute alcohol.

These dextrans were dehydrated from aqueous dispersions (*p*H of 5–6) by lyophilization.

(C). **Heterogeneous Dextrans.**—Some dextran products after precipitation from the supercentrifugate by ethanol in the concentration range 35–42 or 35–45%, were found to be separable into fractions having distinctly different properties.[26] In some cases the whole dextran was characterized (B-742 and -1254; Table I, heterogeneous group), as well as its less soluble and more soluble components, designated by the suffixes -L and -S, respectively. In other cases, only the components fractionated from the dextran were characterized (B-1299, -1355, -1498 and -1501). Complete details on the methods and all the products of fractionation will be reported elsewhere.[26]

Analytical Methods. (A). **Moisture.**—After completion of drying, all dextrans were equilibrated with atmospheric moisture under constant conditions of 21° and 61% relative humidity. The dextrans were stored, and all samples for analyses were weighed, under these same conditions. For determination of moisture content, approximately 0.3-g. samples were held *in vacuo* in an unheated oven for about 16 hours and then heated at 100° and 2 mm. pressure to constant weight (about 30 hours). Moisture contents usually were 14–16%. Dextrans heated in this way have been reported to retain about 0.30% moisture, as shown by use of the Karl Fischer reagent.[40]

All calculations for other analyses were made on a dry basis.

(B). **Periodate Oxidation.**—The types and proportions of anhydroglucopyranosidically linked units (AGU) in the purified dextrans were determined by sodium metaperiodate oxidation.[16,20] Units designated as linked 1- or 1,6-, 1,4-like or 1,3-like reduced 2, 1 or 0 moles of periodate and produced 1, 0 or 0 mole formic acid/mole AGU, respectively. All values reported for water-soluble dextrans are for the 72-hour period of oxidation. Measurements of periodate reduced were made at 25° unless stated otherwise.[20]

Formic acid measurements were precise to ±0.004 mole/mole AGU and accurate to within 1%. The calculation of non-1,6-linked units is based upon measurement of periodate reduced. Measurements made at 25°, such as those in Table I, were precise to ±0.02 mole IO_4^-/mole AGU. However, the percentages of 1,4-like and 1,3-like linked units reported in Table I may be in error by as much as 5%. When the measurements were made at 4°, the error was reduced to 2–3%.[20] This statement of accuracy is based on methylation-structure analysis of the one dextran, B-512, which shows it to have 95% 1,6-linked units and 5% 1,3.[21] Under the conditions of periodate oxidation–reaction analysis by which the data shown in Table I were obtained, this dextran appeared to have 5% 1,4-like linked units and no 1,3-like.

(C). **Specific Rotation.**—Specific rotations were read with sodium vapor light, on solutions filtered through fritted glass when necessary to remove traces of extraneous matter, and are accurate to ±2°. Dextrans were dissolved in ice-cold formamide which had been distilled *in vacuo*. Those of low solubility in water were insoluble in formamide and were dissolved either in ice-cold potassium hydroxide (1 *N*) or were autoclaved in a small amount of water to increase hydration and then made up to 1 *N* with potassium hydroxide solution. Only a few dextrans gave sufficiently clear solutions in water to permit measurement of rotation (examples: B-512, -1072, -1127, -1197).

(D) **Intrinsic Viscosity and k_1 Parameter.**[41]—For measurement of intrinsic viscosity, water-soluble dextrans were dissolved at 4 or 25° and then autoclaved at 15 lb./in.^{2} (120°) for 30 minutes (*p*H 5–6). Water-insoluble dextrans were dissolved in 1 *N* potassium hydroxide solution. Measurements were made on the original solution after pressure filtration through fritted glass and on two other solutions obtained by successive dilutions of the filtered original. These dilutions were made gravimetrically. Measurements were made in No. 100 Ostwald–Cannon–Fenske tubes. The k_1 parameter was calculated from the concentration dependence of the specific viscosity.[42]

(E) **Other.**—Nitrogen analyses were made by the micro-Kjeldahl procedure on 100-mg. samples; phosphorus was determined by a colorimetric method.

Qualitative tests for fructose in dextrans were made by use of 85% phosphoric acid to a limiting value of about 0.2%.[14] Quantitative measurement was made by colorimetric methods.[43]

Results

Data on the purified dextran products and on the strains from which they were derived are shown in Table I.

In Table I, the dextrans are organized into three classes (A, B and C) in which they are arranged in the order of decreasing contents of 1,6-linkages, and increasing contents of 1,4-like and 1,3-like linkages. Structurally heterogeneous dextrans which contained components belonging in different classes are listed separately at the end of Table I.

Products Included in Table I.—With few exceptions the dextrans in Table I comprise major parts of the total fermentation products of the cultures precipitated from the culture supercentrifugates by ethanol in the concentration range 35–50%. These dextrans are designated merely by the strain number, *e.g.*, B-1146. The six structurally heterogeneous dextrans have been subfractionated; in each case data are shown for the less soluble (L) and the more soluble (S) components. A few representatives of the other types of polysaccharide products also obtained from the dextran-containing cultures are included in Table I and are designated by suffixes to the strain number ("I," not in solution in the culture but water-soluble during purification; "A," soluble in the culture but insoluble in the range of ethanol concentration, 0–35%). "A" fractions were always removed from the culture but were not always isolated; only a few of those isolated are reported here. The presence within the same culture of fractions which have the general characteristics of dextrans but which differ physically and/or chemically evidences the molecular heterogeneity of dextran preparations. The phenomenon of molecular heterogeneity of dextran will be discussed more completely elsewhere.[26]

Yields.—The highest yield of purified dextran product, based on the weight of sucrose in the culture, was 29%. This is 61% of the theoretical. Losses during purification usually were about 3–5%. The average yield of the products listed in classes A, B and C were 16, 11 and 13%, respectively.

Our objective was to obtain representative dextrans of highest purity, rather than to develop conditions for maximal yields. Attention to individual culture requirements doubtless would improve the yield from many strains.

Composition of Purified Dextrans.—The purified dextran products contained no more than about 0.02% fructose, which is the limiting value of the colorimetric method of analysis employed.[43] Dextran B-1351 was the sole exception[35]; it contained 0.26% fructose which has been shown to be a constituent of the dextran.[44]

Glucose was the only sugar found by paper chromatography of a few dextrans of special inter-

(40) R. L. Weiman, R. H. Cundiff, T. C. Yao, E. E. Toops, Jr., and J. A. Riddick, *Abstracts Papers Am. Chem. Soc.*, **122**, 15A (1952).

(41) We are indebted to Dr. N. N. Hellman for planning the procedures for measurement of intrinsic viscosity and for calculation of k_1 values.

(42) (a) R. Simha, *J. Research Natl. Bur. Standards*, **42**, 409 (1949); (b) *J. Colloid Sci.*, **5**, 386 (1950).

(43) C. S. Wise, R. J. Dimler, H. A. Davis and C. E. Rist, *Abstracts Papers Am. Chem. Soc.*, **124**, 2D (1953); *Anal. Chem.*, in press.

(44) E. J. Hehre, private communication.

est (B-512, -523 and -742) after complete acid hydrolysis.[45]

Percentages of nitrogen, phosphorus and ash did not exceed 0.01, 0.003 and 0.05, respectively, and often were less in both water-soluble and water-insoluble dextrans.

Character of Dextran Precipitates and Solutions.—The nature of the dextran product was observed after precipitation of the dextran from aqueous solution by ethanol of 45–50% concentration and during washing with 50% ethanol (Table I). The products were either gums, or flocculent or fine powders. The gums were either "long" (that is, under gentle tension they showed elasticity or ability to flow rather than breaking apart) or "short" (that is, under slight tension the gum broke apart readily). All gradations of these types were found. Thus, the "stringy" gums pulled out to fine threads but showed less tendency to stream in large masses than did the "long" gums. Some of the stringy gums were fluid or sirupy.

The dextrans were isolated in such ways that molecular aggregation did not contribute significantly to insolubility.[15] The ease of solubility of the dextran was, therefore, a characterizing property. Dissolution of dextrans in water, formamide or dilute alkali occurred much more readily at about 4° than at 25°. Heating dextrans of low solubility usually was ineffective unless dispersion already was essentially homogeneous. Many dextrans in classes A and B dissolved readily in concentrations up to 30–40% merely by adding water (examples: B-1146, -512, -1397 and -641). Other dextrans appeared to be insoluble unless water was worked in gradually to allow all particles to become dispersed in their own dense paste (examples: B-1064, -1066, -1382, -1383, -1308 and -1255). The majority of dextrans in class C and in the heterogeneous group were much more difficult to dissolve than those of class A and became progressively more so as the content of non-1,6-linkages increased.

The dense (40–50%) aqueous dispersions of most dextrans were brilliantly clear; some notable exceptions were dextrans B-1196, -1405, -1407, -1414 and -1419. Dilute aqueous solutions (2–5%) of the long and stringy gums were clear or slightly opalescent. With a few exceptions all others were turbid to varying degrees.

Discussion

General Aspects of Survey.—The 96 bacterial strains used for production of the dextrans described here came from 5 genera and constitute the individual strains from a total of 135 examined. Although the strains were from a variety of natural sources,[24] the sampling was not sufficiently large or diverse to assure inclusion of all possible dextran-producing types. Sufficient correlation has not been found between strain classification or origin and dextran characteristics to provide a basis for classification of the dextrans.

The assumption may be made with reasonable assurance that the one constant feature of all these dextrans is their structural component which appears to be almost exclusively the anhydroglucopyranose unit of alpha configuration. The other chemical and the physical characteristics of the dextrans cover wide ranges of values, as is shown in Table I. The 1,6-glucosidic linkages constitute from 50–97% of the total linkages. As determined by periodate oxidation–reaction analysis, the non-1,6-linkages are of two types, the 1,4-like and the 1,3-like. Either type may constitute all or only part of the total non-1,6-links. The intrinsic viscosity, solubility and nature of the dextran precipitates, like all the other dextran properties, constitute continuous spectra of values having small increments of variation, yet differing widely in the extremes. However, definite natural types among the dextrans are indicated by the recurrence of certain combinations of properties in dextrans from different strains.

Classification of Dextrans.—From the different types and proportions of glucopyranosidically linked units present in the various dextrans, as determined by periodate oxidation, it is clear that a great range of structures is represented in the dextran class of polysaccharides. A convenient separation of the dextrans into three classes has, nevertheless, been made using the periodate oxidation–reaction data (Table II). The differentiation of these classes depends primarily upon the content of 1,3-like linked units. The demarcation in content of 1,3-like linked units for class A was based upon the limit of positive detection, 2% instead of 0%. For class B, the actual proportions of the 3 types of links in the dextrans determined the point of division. Class C dextrans, with a few exceptions, had significantly higher contents of 1,3-like links or lower contents of 1,6- than those of class B. Those dextrans found to be separable into components belonging in different classes have been grouped separately. Data on these dextrans and/or their major components are shown at the end of Table I. Although at present, dextrans from only 6 strains have been shown to be structurally heterogeneous, we believe that further investigation of a number of other dextrans (such as B-1142, -1192, -1255, -1351 and -1374) would reveal a similar complexity.

TABLE II

CLASSIFICATION OF DEXTRANS

	Carbon atoms of AGU involved in glucopyranosidic linkages			
	C_1 (C_1 and C_6)	C_1 (C_1 and C_6)	C_1 (C_1 and C_6)	
Designation[a]	and C_4-like	and C_3-like	No. of dextrans	
Class	1,6	1,4-like	1,3-like	
A	97–50	0–50	0–2	47
B	95–86	0–8	3–6	15
C	85–50	0–36	>6	28
Molecularly heterogeneous dextrans which have been fractionated into major components belonging in 2 or more of the other classes, respectively.				6

[a] The significance of the term, "like," has been stated previously.[20]

The lowest value for 1,4-like linked units in dextrans of classes A and B, 2% (Table I), is within the precision of our analytical procedure. Furthermore, as has already been shown for B-512 dextran, other dextrans in these classes might appear by periodate oxidation procedures to have higher contents of

(45) R. J. Dimler, H. A. Davis, G. J. Gill and C. E. Rist, unpublished data.

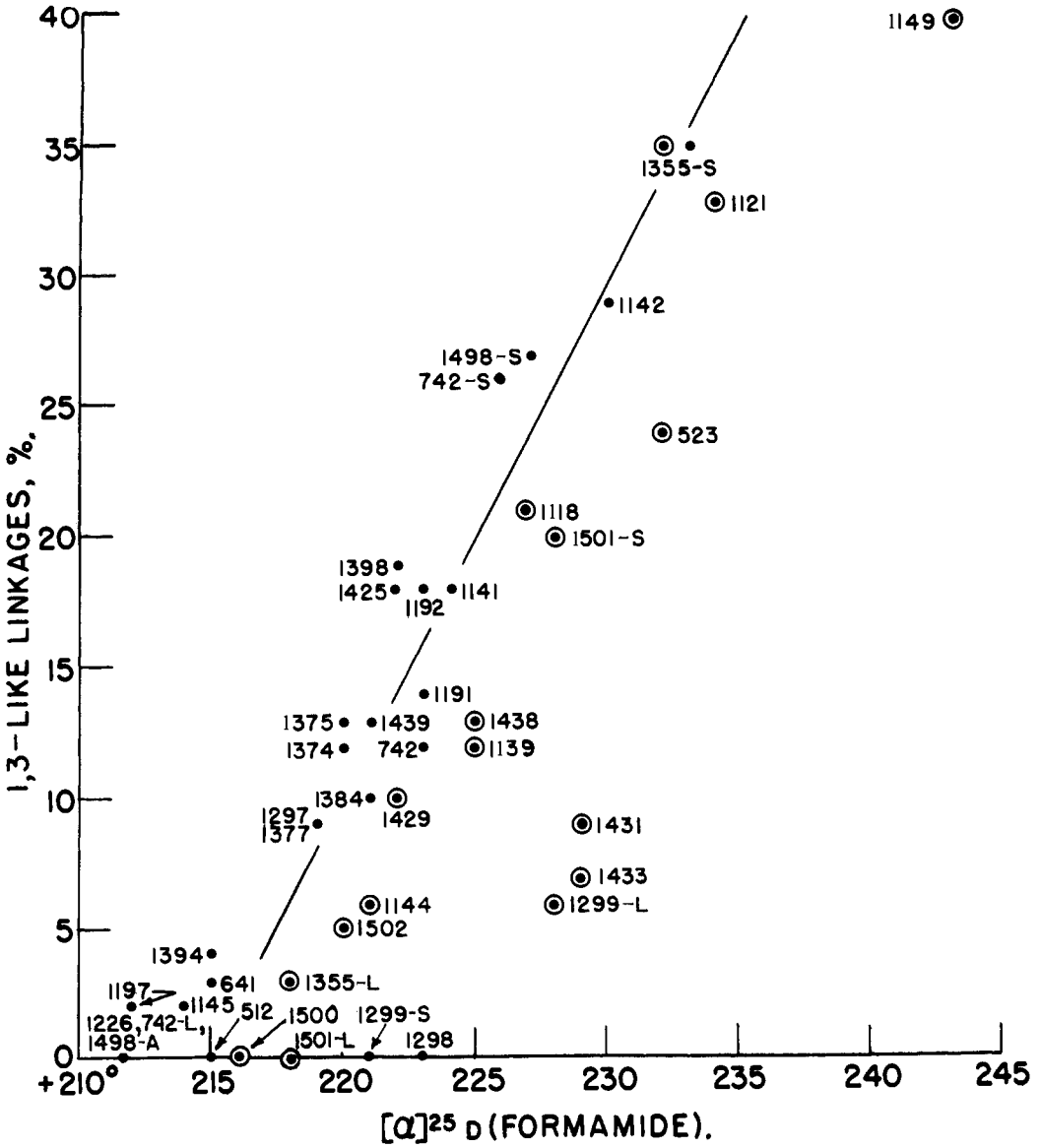

Fig. 1.—Correlation between specific rotation and content of 1,3-like linkages in dextrans: ●, rotation observed in formamide; ⊙, rotation observed in 1 *N* potassium hydroxide and calculated to formamide by assuming that all dextrans show the same difference in rotation in these solvents as do dextrans B-512 and B-1355S.

these links than are actually present. Therefore, the lower limit for 1,4-like links is indicated as 0% in Table II.

This mode of classification, although restricted by the limitations of the periodate oxidation method of structural analysis,[20] is adequate for the present and permits extension in the future. Specific identification of the non-1,6-linkages will permit more accurate classification of the dextrans. As will be shown in a later section, the type of link designated 1,3-like already appears to include more than one kind of structure. The same might also be found true for the 1,4-like type.

Correlation among Dextran Properties

Linkages.—The arrangement of the dextrans within the classes in the order of decreasing content of 1,6-links and increasing content of the non-1,6 is suggestive of an order of increasing degree of branching. However, the periodate data provide no direct evidence for this interpretation. Non-reducing end groups are not differentiated from units within the chain linked through C_1 and C_6. The non-1,6-linked units merely indicate the maximum proportion of branch points possible, but some or all of them may be units within a main, straight chain. However, methylation data for B-512 dextran[21] and for several other dextrans,[11,13] prove the non-1,6-linked units to constitute branch points.

Specific Rotation.—The specific rotation of dextrans differs with the solvent.[46] Thus, B-512 dextran showed values of 199, 203 and 215° in water, 1 *N* potassium hydroxide and formamide, respectively. Rotations in formamide ranged from 208

(46) All rotations referred to are positive and were obtained in formamide unless stated otherwise.

to 233° for the different soluble dextrans, and from 203 to 232° for those dextrans tested in potassium hydroxide. Though not recorded in Table I, rotations in water were measured for a few dextrans, with 196° the lowest value observed. None of our dextrans had specific rotations near those of 180° in water[8,10,13] and 180–190° in alkali[6,9,10,13] reported for some dextran preparations.

The rotation, 215 ± 2°,[46] was characteristic of the great majority of class A dextrans which had more than 75% 1,6-linkages. Thus, variation of 1,6-linkage content between 97 and 76% and of the 1,4-like between 2 and 24% did not appear to influence the rotation. This might have been expected from the fact that the specific rotation of starch in water, +200°, is essentially the same as that for B-512 dextran. Dextrans of classes B and C had no characteristic rotation, but usually the rotation increased proportionally with the content of 1,3-like linked units as is shown in Fig. 1.

However, class A dextrans having 1,4-like linkages ≧25% (excepting B-1399 dextran) showed rotations significantly higher than 215°. This was true also of the similarly constituted fractions from B-1299 (heterogeneous group) (Fig. 1). It appears that the high rotations of these dextrans and dextran fractions are related to the presence of 1,3-like linked units which, as is shown in the following section, have been detected by infrared absorption analysis but not by periodate oxidation. There is good agreement between the rotations and the contents of 1,3-like linked units estimated from the infrared spectra of all these preparations except B-1298 and B-1299L. For these two substances, the rotations are so high as to suggest the influence of some additional and unidentified factor.

Rotations significantly lower than 215° were shown by a few class A dextrans. Dextran B-1197, with rotations 212 and 196° (water), is known to contain some 1,3-like linked units but no detected fructose. Dextrans B-1225 and B-1226, which were produced from dextrin by *Acetobacter* species, showed rotations of 208 and 212°, respectively. Our rotation for the B-1225 dextran is in agreement with that previously reported by Hehre.[19b] This dextran, as well as the dextran fraction, B-742L (heterogeneous group) which also showed a rotation of 212°, was outstandingly free of 1,3-like linked units. Absence of this type of link might also account for the rotations, 211–212°, shown by several other dextran fractions.

Infrared Spectra.—Infrared absorption analysis provides further evidence of the presence of a distinctive structure in dextrans which show 1,3-like links by periodate analysis and/or rotations higher than about 212 ± 2°. Dextrans were differentiated by Burket and Melvin as "Type I" and "Type II" according to whether they showed little, or appreciable, absorption at 12.6 μ.[47] From the infrared absorption spectra of dextrans, Melvin, *et al.*, now have calculated the percentage of Type II structure present[48] and from these values the percentages of 1,3-like links in the dextrans have been estimated. These procedures utilized the facts that our dextran preparations, B-742L, which shows no 1,3-like linked units by periodate analysis, and B-1355S, which shows the highest content of these links by periodate analysis of any of our products, are models of the infrared Types I and II, respectively.

In Table III are shown some representative values of 1,3-like linked units obtained by periodate oxidation and by infrared analysis. These values establish direct, quantitative relationship between the contents of Type II and of 1,3-like linked units in most dextrans.

TABLE III

CONTENT OF 1,3-LIKE LINKED UNITS IN DEXTRANS, CALCULATED FROM PERIODATE OXIDATION AND FROM INFRARED ABSORPTION DATA

Dextran NRRL B-		1,3-Like links, %: Periodate[a]	Infrared, Type II
A	1146	0 (3)	3
	512	0 (3)	5
	1308	0 (2)	3
	1197	2	2
	1225	0	0
	1383	0 (0)	4
	1382	0 (1)	2
	1424	0 (0)	5
	1402	0 (0)	5
	1298	0 (0)	6
B	1193	3	4
	641	3	2
	1387	3	4
	1419	3	5
	1255	4	4
C	1351	11	11
	1139	12	9
	1385	10 (13)	14
	1118[b]	21	5
	1192	18	18
	1191	14	18
	523[b]	24	2
	1121[b]	33	11
	1433[b]	7	5
	1149[b]	40	0
Heterogeneous-dextran group			
	742L	0 (0)	0
	742S	26	27
	1299L	6	7
	1299S	0 (0)	9
	1355S	35	35

[a] Values in parentheses were obtained by making the iodimetric titration for periodate reduced at 4° instead of at 25°.[20] [b] This is one of the dextrans which showed enhanced infrared absorption at 10.6 and 12.2 μ.

Most of the class A dextrans listed in Table III are ones whose percentages of 1,3-like linked units as determined by periodate analysis appeared low; others not listed also showed detectable Type II absorption. For class A dextrans having more than 75% 1,6-linked units, agreement between periodate and infrared analyses was within the precision of the methods, especially if titration for periodate was made at 4°[20] (Table III). For most class A dextrans having 75% or less 1,6-links (B-1397, -1424, -1402 and -1298) the differences were greater

(47) S. C. Burket and E. H. Melvin, *Science*, **115**, 516 (1952).
(48) E. H. Melvin, *et al.*, data in preparation for publication.

and were not eliminated by 4° titration. This is true also of dextran fraction B-1299S, but not true of B-1299L (heterogeneous group).

With a few exceptions, the dextrans of class C and the heterogeneous group showed good agreement between contents of 1,3-like linked units as shown by periodate and by infrared analyses (Table III). The main exception was the water-insoluble dextran, B-1149, which showed no Type II absorption, but slightly greater absorption at 10.6 and 12.2 μ than did dextrans of Types I and II. Several other water-insoluble dextrans (B-1121, -523, -1118, -1433 and -1431) showed this new absorption in decreasing amounts and in addition to some Type II absorption (Table III). The rotation of most of these dextrans was higher than would be expected from periodate analyses (see Fig. 1).

The correlation among data from periodate oxidation, optical rotation and infrared absorption appears to indicate that in most dextrans the 1,3-like links are identical; possibly they are the 1,3. However, a different structure not distinguishable from the 1,3 by periodate oxidation appears to be present in a few dextrans.

Intrinsic Viscosity.—The intrinsic viscosities[49] show a wide range of values in each class and an over-all range of 0.15–2.0. Even the highest of these viscosities are relatively low for polymers of such high molecular weight. In both classes A and C, the maximum in the number distribution of intrinsic viscosities was in the range 0.85–1.10. In class A, there were more dextrans of viscosity below this range than above it, but the reverse was true for class C dextrans.

No consistent trend appears in the viscosity values[49] as the proportion of 1,6-linked units decreases (Table I). If it be assumed that this decrease corresponds to increased branching and that the frequently observed proportionality between linearity and intrinsic viscosity be applicable to these dextrans, a trend toward lower viscosities should be evident. Likewise contrary to the observations, the distribution of viscosities in classes A and C would be expected to conform to this proportionality between linearity and viscosity. However, the influence of branching might be obscured by variations in particle weights.

There appears to be rough proportionality between the content of 1,6-links and viscosity in the series of fractions from dextrans B-1254, -1355, -1498 and -1501, respectively (Table I, heterogeneous group). However, this is not true for the B-742 fractions. The known particle weight of B-742L is so high as to preclude size as the cause for its exceptionally low viscosity.[50]

These observations permit no generalizations concerning the influence of the type and proportion of 1,6-links on the viscosity of dextrans in water.

The highest viscosities obtained were for water-insoluble dextrans in 1 N potassium hydroxide (B-523, -1433 and -1431; Table IC). There is insufficient information to establish a positive role for the solvent in producing these high viscosities. The fact that for a few water-soluble dextrans the intrinsic viscosities in 1 N potassium hydroxide normally were only 1.2–1.4 times those in water,[51] would indicate a value in water of about 2.2–1.9 for dextran B-1433. This is significantly higher than the intrinsic viscosities for any of our dextrans excepting B-1394 (class B).

Shape Factor—Gum Properties.—The concentration dependent parameter of viscosity, k_1, which is shown for the dextrans in Table I, was defined by Simha and shown theoretically to depend in a characteristic manner on particle shape, the solvent environment and molecular weight.[42] Branched molecules appear to have higher k_1 values than linear molecules.[42b] Values in the range 0.36–0.77 were derived for molecules of lowest density, such as those thread-like or randomly coiled in shape, and 0.64–2.24 for the densest or spherically shaped ones.[42]

Specific deductions concerning particle shape cannot be based on the values reported here since several factors which influence the k_1 parameter have not been measured for our dextrans. However, for this extensive series of polysaccharides, it seems justifiable to point out that the k_1 values are all within the limits calculated by Simha, and that definite relationships exist between k_1 and certain other data for the dextrans.

The k_1 values for our dextrans make up a continuous series. In this series, the dextrans do not occur randomly, but group themselves at definite positions according to their percentage of 1,6-links and to the characteristics of their gums or precipitates (Fig. 2). The boundaries of the groups encompass dextrans having comparable gums or precipitates (Table IV), and were drawn arbitrarily to show the relationships most simply. Groups have been subdivided when dextrans of class C showed k_1 values and properties comparable with those of classes A and B (groups 3a, 3b and 4a, 4c) or when dextrans within classes A and B showed significantly different properties (groups 4a, 4b and 5a, 5b).

Dextrans in groups 2, 3a, 4a and 4b show very homogeneous and distinctive properties, respectively, as well as relatively narrow ranges of intrinsic viscosity (Table IV). Group 2 is composed almost exclusively of fractions of dextrans having the lowest of all our viscosities. Dextrans of groups 5a and 4c show wide ranges in viscosity and several types of gums. Several of the dextrans in 5a had short gums, but others showed stringiness (B-1409, -1415, -1392 and especially -1127). In group 4c there are gums which were long (B-1377), stringy (B-1374 and -1424), fluid and stringy (B-1425, -1439 and -1525), as well as short. All the dextrans having long or stringy gums had k_1 values in the range 0.70–1.1, and all except one in the range 0.80–1.1.

Most of the other dextrans listed in Table I but not included in Fig. 2 also fit into one of the established groups. In several cases, lack of conformity revealed the k_1 value to have been in error. Several other values appear questionable since their dextrans showed no characteristics of the groups in which they would fall (for example, B-1414 in 4a

(49) Intrinsic viscosities in water are referred to unless stated otherwise.

(50) N. N. Hellman, private communication.

(51) J. E. Cluskey, R. J. Dimler, B. E. Fisher and C. E. Rist, *Abstracts Papers Am. Chem. Soc.*, **124**, 4D (1953).

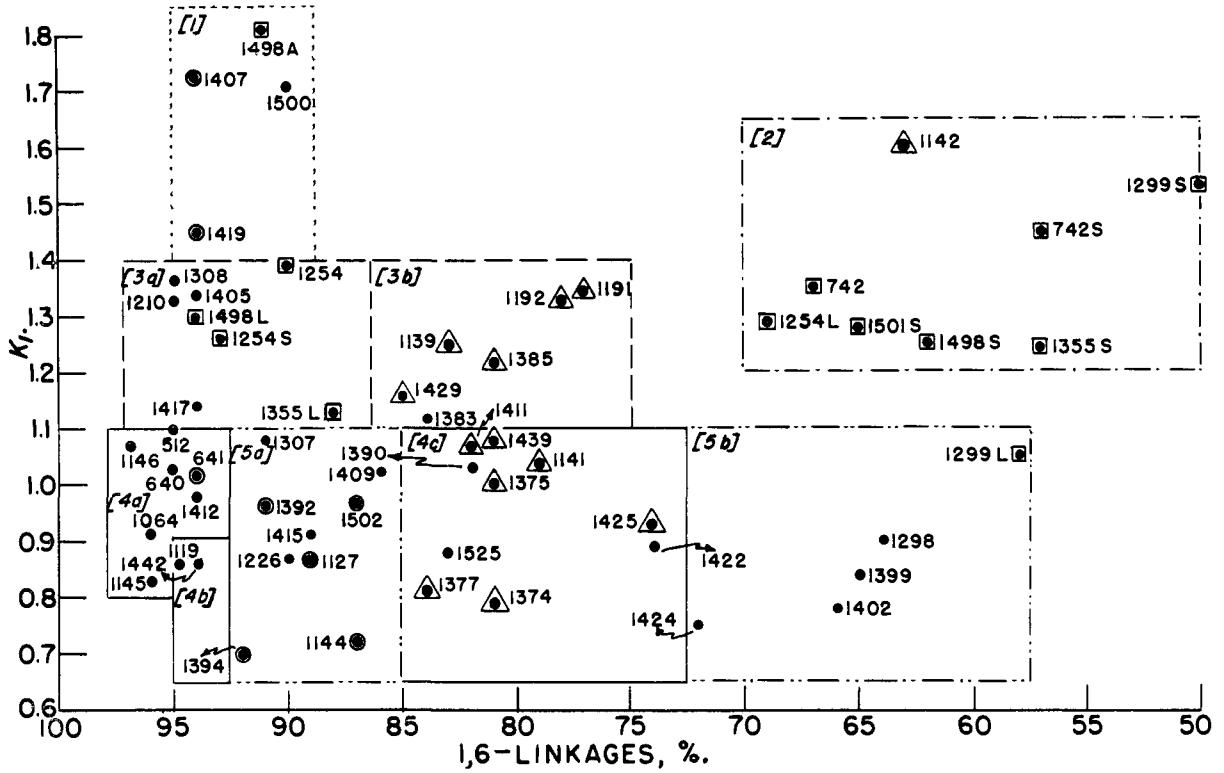

Fig. 2.—Relationships among k_1 parameter of water-soluble dextrans, per cent. of 1,6-links, and nature of the dextran precipitates.[a] The classification of the dextrans is indicated by: ●, A; ⊙, B; △, C; ⊡, heterogeneous dextran group. The key to the group designations is given in Table IV.

[a] For a few dextrans, the properties of the precipitate agreed better with those of an adjacent group than with those of the group where plotted. This is indicated by an arrow from the plotted point to the dextran number.

and B-1443 in 4c). However, we do not wish to imply the expectation that the group demarcations in Fig. 2 will hold rigidly for all dextrans. Even for our own different preparations of dextran from strains B-512, -742 and -1146, respectively, the k_1 values showed a variation of about ±0.10.

Relationships between the Strains and Dextran Properties. Constancy and Reproducibility of Dextran Properties.—We have found that for many dextrans apparently exact reproduction of results was obtained in repeated preparations and that, for many of the strains, modifications such as those indicated in the Experimental section could be made in culturing conditions without detectable changes resulting in the dextrans.[24] Under other conditions, dextrans from a few strains have shown definite variations which might have resulted from more critical modifications in culture conditions or from changes in the culture itself or from differences in fractionation during purification of the dextrans. Thus, our first preparations of B-523 and of B-742 dextrans gave evidence of little or no 1,3-like links,[16] in contrast to the much higher contents of the corresponding preparations reported here.

The structure of our first preparation of B-742 dextran was in excellent agreement with that indicated by methylation-structure analysis on dex-

TABLE IV

CORRELATION OF DEXTRAN PROPERTIES WITH DATA SHOWN IN FIG. 2

Group	Class of dextrans[a]	Nature of dextran precipitate[b]	[η] (water, 25°)	Appearance of dilute aqueous soln.[c]
1	A, B	V. cohesive, tough, short gum	1.16–0.57	V. turbid
2	A, C	Fine or floc. ppt.	0.47–0.19	Marked bluish opalescence
3a	A, B	Fine or floc. ppt. to crumbly or dense short gum	0.89–0.47	V. turbid
3b	C	Floc. ppt. to crumbly or dense short gum	1.36–0.50	V. turbid
4a	A, B	Long, soft gum	1.28–0.95	Sl. opalescence
4b	A, B	Uniquely cohesive, stringy gum	2.00–1.62	Sl. opalescence
4c	A, C	Long, stringy or fluid gums	1.36–0.42	Clear to sl. turbid
5a	A, B	Short or stringy gums	1.20–0.56	Turbid or sl. opalescence
5b	A, B	Floc. ppt. or short gum	1.03–0.87	Sl. to v. turbid

[a] Dextrans or their components from the heterogeneous group are included in their respective classes. [b] Observed when precipitated from aqueous solution by ethanol of 45–50% concentration. [c] Approximately 1–2% concentration.

tran from this same strain.[16,52] Our subsequent preparations from this strain have contained 2 distinct structural types of dextran, one of which (fraction L or L-R) apparently was identical with previous preparations and the other (fraction C or S-R)[26] was an entirely new type having a high content of 1,3-like links. Characterization of dextran products from 12 different colonies picked from a plated culture of B-742 failed to reveal evidence of variation or mutation in the culture. These products had somewhat different proportions of the 2 structural types, 1,6-links from 72–75% and intrinsic viscosities near 0.20. The preparation reported in Table I (heterogeneous group), which was obtained from a large-scale fermentation of the original culture, had a higher content of the anomalous fraction and, therefore, showed lower 1,6- and higher 1,3-like linkage contents and higher viscosity and rotation.

The strains B-742 and B-1142 (class C) had a common origin but different subsequent histories. Our B-1142 dextran consisted almost exclusively of the anomalous fraction. Apparently these cultures have been changing, B-1142 more than B-742.

(52) Published information[11] was the basis for our identifying strain NRRL B-742[16] ("number 5" of Hucker[17] and "culture 4" of Tarr and Hibbert[4]) with the strain whose dextran was subjected to methylation-structure analysis by Levi, *et al.*[11] Another source (Ph.D. thesis of I. Levi, McGill University, 1942) now has disclosed that this methylation study was made on dextran from either "culture 1" or "culture 2."[4] Fowler, *et al.*,[5] and later T. H. Evans (Ph.D. thesis, McGill University, 1941), carried out methylation-structure analysis on dextran from "culture 4" but obtained results almost identical with those obtained by Levi, *et al.*, on the other dextran.[11]

A similar change in another strain is indicated by published data[13,53] and by our results on dextran from this strain (B-1375, Table I, class C).

Bacterial Classification and Dextran Type.—The fact that methylation analysis gave no evidence of branching in dextrans from 2 strains of *Leuconostoc dextranicum*[7,8,13] has led to the apparent expectation that all such strains would produce essentially a straight-chain type of dextran.[54] Our observations indicate that although several of our strains of *L. dextranicum* produced dextrans with low percentages of non-1,6-links and long gums (B-640, -1145 and -1146), others produced dextrans with high percentages of non-1,6-links and short gums (B-1420, -1141 and -1375). Dextran B-1193 had 95% 1,6-links, but short gum.

Acknowledgment.—We should like to express our gratitude to the numerous individuals and organizations named in Table I who gave us cultures utilized in this investigation. We are deeply indebted to Dr. E. J. Hehre for advice and encouragement and to members of this Laboratory, as follows: Dr. R. T. Milner for coördination of this project; C. H. vanEtten, T. A. McGuire and Mary Weile for the nitrogen, phosphorus and ash analyses; and Lenora J. Rhodes and Geraldine Bryant for assisting with production of the cultures and of the media, respectively.

(53) S. A. Barker, E. J. Bourne, G. T. Bruce and M. Stacey, *Chemistry and Industry*, 1156 (1952).

(54) S. A. Barker and E. J. Bourne, *Quart. Revs. (London)*, **7**, 56 (1953).

PEORIA, ILLINOIS

[CONTRIBUTION FROM HARRIS RESEARCH LABORATORY AND NATIONAL RESEARCH COUNCIL]

Biosynthesis of C^{14}-Specifically Labeled Cellulose by *Acetobacter xylinum*. II. From D-Mannitol-1-C^{14} with and without Ethanol[1]

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Cellulose-C^{14} was biosynthesized by *Acetobacter xylinum* employing D-mannitol-1-C^{14} as the sole labeled nutrient. Label distribution in the D-glucose from the bacterial cellulose showed 84–96% of the activity was located at positions 1 and 6, with the residual activity being at positions 2 to 5. These data indicate that some of the scission products from the original D-Mannitol became oriented in the cellulose. The labeled cellulose had lower specific radioactivity than the D-mannitol-1-C^{14} supplied. The presence of ethanol in the culture media, although it increased the yield of cellulose as well as its C^{14} content, did not affect the distribution of the label among the carbons of the glucose making up the cellulose.

Introduction

This paper is the second in a series concerned with the ability of *Acetobacter xylinum* to produce cellulose-C^{14} from various substrates, possibly providing some information regarding the mechanisms involved in cellulose formation. Presented are results of experiments in which D-mannitol-1-C^{14} was the sole labeled additive to a medium suitable for the organism to produce cellulosic pellicles. Analyses reported include the distribution of the C^{14}-label among the various culture products as well as the spread of radioactivity in the purified glucose from the hydrolyzed C^{14}-cellulose.

(1) This series of papers is based on work supported by the Atomic Energy Commission under contract AT(30-1)-915 with the Harris Research Laboratories.

(2) National Research Council, Washington 25, D. C.

Experimental

Culture Conditions.—The cultures were grown in media containing 0.3% of KH_2PO_4 plus other ingredients, as shown in Table I. Each culture was inoculated from actively growing stock. Culture 5 was incubated at 30° and harvested 7 days following inoculation; while the others were grown at room temperature (20 to 25°) and harvested 14 days after inoculation. The culture vessel, including accessory apparatus for collecting CO_2, was the same as that previously described.[3]

Analyses.—The methods for purifying the cellulose and for determining the C^{14}-content of culture fractions were identical with those described in the preceding paper of this series.[3] Except for the procedure involving lactic acid

(3) F. W. Minor, G. A. Greathouse, H. G. Shirk, A. M. Schwartz and M. Harris, THIS JOURNAL, **76**, 1658 (1954).