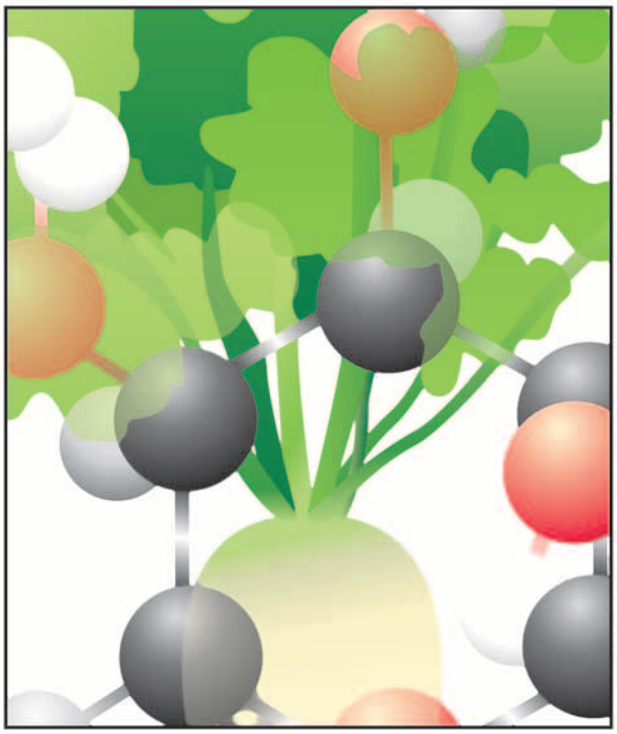
# Dextran



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# Dextran

by A.N. de Belder

# Contents

# 1. Introduction

A brief search of the Chemical Abstracts database for dextran and dextran-related titles leaves one in little doubt of the prolific interest in this area; an interest which may be attributed to the continuing clinical, scientific and technical importance of dextran and its derivatives.

Currently, more than 1000 publications dealing with dextran appear each year. Reviews in this field are published with impressive regularity (1-25).

Certain limitations had to be imposed on this Review and it has thus been restricted to dextrans of commercial interest, in particular from *Leuconostroc mesenteroides* NRRL B-512(F); other dextrans and the cariogenic glucans are only referred to where they are of particular relevance. References to dextranases will be confined solely to those of interest to the B-512(F) dextran.

Certain abbreviations have been employed throughout:

| | |
|---|---|
| MWD | molecular weight distribution |
| $\overline{M}_W$ | mass average molecular mass |
| $\overline{M}_n$ | number average molecular mass |
| MW | molecular weight |
| Dextran 70 | a dextran fraction with $\overline{M}_W$ of 70 000 |
| DS | degree of substitution |

# 2. Structure, physical-chemical properties and reactivity

## 2.1. Structure

The dextran elaborated by *Leuconostoc mesenteroides* NRRL B-512(F) consists of an α(1→6)-linked glucan with side chains attached to the 3-positions of the backbone glucose units. From periodate (26, 27) and methylation (28–31) analyses, the degree of branching is estimated as 5%. The value of the methylation analysis is that it not only identifies the (1→3) branch linkages but also quantitates them. A systematic study of this analysis in our laboratories, however, has revealed that the estimation of 2,4-dimethyl-glucose by gas-liquid chromatography is subject to a rather high coefficient of variation; 11-20% depending on the conditions used. The degree of branching is found to decrease on partial acid hydrolysis, although the effect is not dramatic (29).

| Dextran | Branching (%) |
|---|---|
| Native dextran | 4.6 |
| Dextran 80 | 3.8 |
| Dextran 10 | 3.0 |

**Table 1.** Dependence of degree of branching on molecular weight.

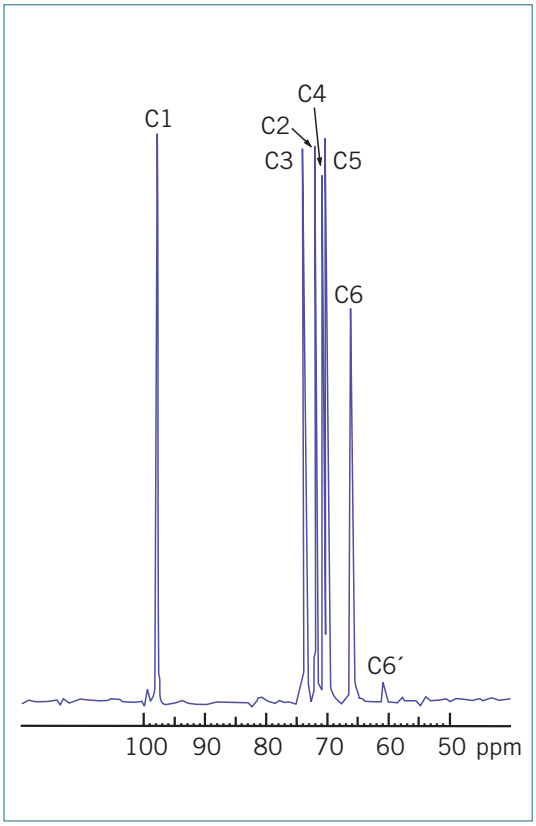



**Fig 1.** $^{13}$C-NMR spectrum of native dextran from *Leuconostoc mesenteroides* B-512(F) in $D_2O$.

Although the values given in Table 1 are somewhat lower than those generally accepted now for B-512(F), they provide a useful relative comparison.

The rationale for this decrease is based on the greater lability to acid of α(1→3) linkages compared with α(1→6) linkages (32, 33).

Using less sensitive techniques, Bremner (34) did not find any difference in the branching between native dextran and dextran, $\overline{M}_W$ 3 000.

The $^1$H- and $^{13}$C-NMR spectra afford compelling evidence for the main structural features of dextran. The $^{13}$C-NMR spectrum for native dextran NRRL B-512(F) is shown in Figure 1 and the individual assignments are shown in Table 2.

| | $^{13}$C (ppm) | $^1$H (ppm) |
|---|---|---|
| C1-H | 97.8 | 4.99 |
| C2-H | 71.5 | 3.6 |
| C3-H | 73.5 | 3.76 |
| C4-H | 70.0 | 3.52 |
| C5-H | 70.3 | 3.88 - 4.04 |
| C6-H | 66.1 | 3.88 - 4.04<br>3.81 - 3.86 |
| C6'-H | 61.0 | — |

**Table 2.** $^{13}$C and $^1$H chemical shifts for dextran B-512(F) (35-37)

The signal at 61 ppm, assigned to the C6 atom on the non-reducing glucose-units, is of considerable interest as it corresponds to the branching (35-36). The downfield signal at 99.5 ppm is tentatively assigned to the α(1→3) anomeric carbon (36). Studies in our laboratories on the determination of the degree of branching of clinical dextran by NMR yields a value of 4.8–5.5 %, depending on the integrating technique employed (38).

Since the early studies on dextrans by Pasika and Cragg (39) using $^{1}$H-NMR, rapid progress has been made in resolution and quality of the spectra. A thorough reexamination of the technique applied to dextrans has been published by Gagnaire (37) and Seymour (40). The assignments for the proton signals are included in Table 2. Since the spectra are necessarily much more complex, it has not been possible to resolve and identify all signals, particularly the minor ones. We have found NMR spectroscopy useful for detecting traces of levan in native dextran. Figure 2 shows the $^{13}$C-spectrum of native dextran containing 20% added levan. Many details of the fine structure remain unresolved, in particular the length and distribution of the side chains.

Sequential degradation, which quantitatively eliminates non-reducing end groups, has been employed to investigate the length of the side chains (30). The procedure is based on the alkaline degradation of terminal glucose units in which the 6-hydroxyl group is substituted with C-p-tolylsulphonylmethyl groups. Larm and colleagues (30) thereby concluded that 40% of the side chains are one unit long, 45% are two units long and the remaining 15% longer than two. Previous studies involving catalytic oxidation had also implied the presence of one unit branches (29).
The preponderance of single unit branches has been demonstrated in several other dextrans by Abbott (41) and Bourne (42). The availability of enzymes with well-characterized properties has permitted Walker and her colleagues to corroborate these results (43, 44). By means of HPLC, she has successfully isolated and identified oligosaccharides with single and double unit branches, for example, 1 and 2, which were obtained by treating native dextran with the fungal dextranase from *Penicillium funiculosum*.

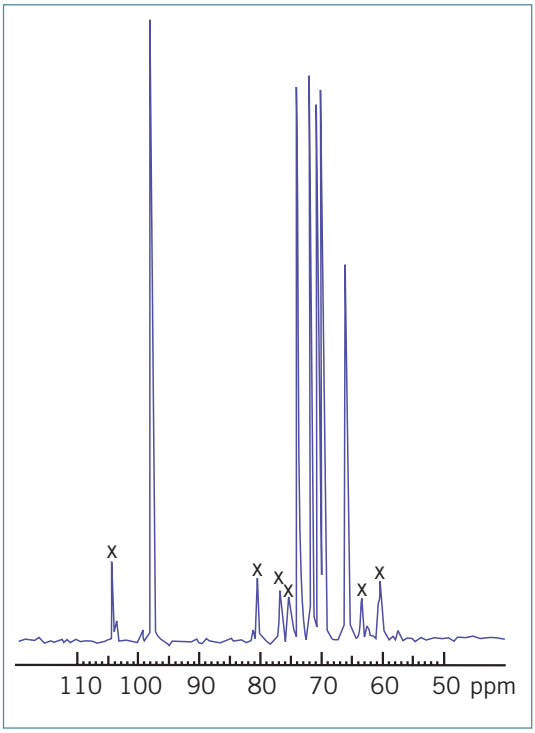
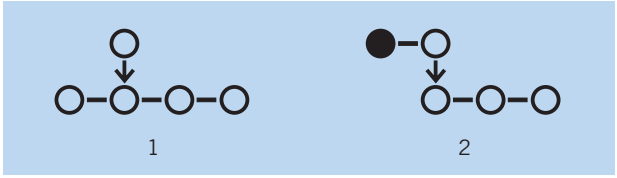



**Fig 2.** $^{13}$C-NMR spectrum of native dextran B-512(F) containing 20% levan in $D_2O$. The signals marked X are due to levan.




$3^{3}$-glucosylisomaltotetraose $3^{3}$-isomaltosylisomaltotriose

A dextran-glucosidase (from *Streptococcus mitis*) with exo-dextranase activity surprisingly released 25% glucose from native dextran B-512(F) (43). The limit dextran obtained had a $\overline{M}_W$ of 20 x $10^6$ and the branching was unchanged indicating no internal cleavage. To account for this amount of glucose, the side chains longer than two units would need to be at least 33 units long. Our understanding of the distribution of side chains is also imperfect. Covacevich and Richards (45) by analysing the distribution of oligosaccharides from the hydrolysate of dextran with an endo-dextranase concluded that the branches were not clustered but were distributed in a relatively regular manner. These conclusions were based on a number of assumptions, one of which, that the branches are only one unit long, is questionable. Evidence for the presence of long branches has also been adduced from studies on the $\overline{M}_W$ and viscosity during the biosynthesis of dextran (46). Further reference to branching is made in the following section.

## 2.2. Physical-chemical properties

Dextran B-512(F) is freely soluble in water, methyl sulphoxide, formamide, ethylene glycol, glycerol, 4-methylmorpholine-4-oxide, and hexamethylphosphoramide (a carcinogenic). Some dextran fractions may adopt a certain degree of crystallinity and may only be brought into solution by strong heating.

The molecular weight of native dextran NRRL B-512(F) has been investigated in many laboratories (46-51) and values for the $\overline{M}_W$ from 9 x $10^6$ to 500 x $10^6$ have been obtained. Measurements in a variety of solvents, for example 4 M NaCl and 6 M urea, failed to reveal any evidence that association contributed to these exceedingly high values (49, 50). The relationship between $\overline{M}_W$ and intrinsic viscosity [η] has been investigated over a wide range of $\overline{M}_W$ (see Figure 3) (47, 52–56).

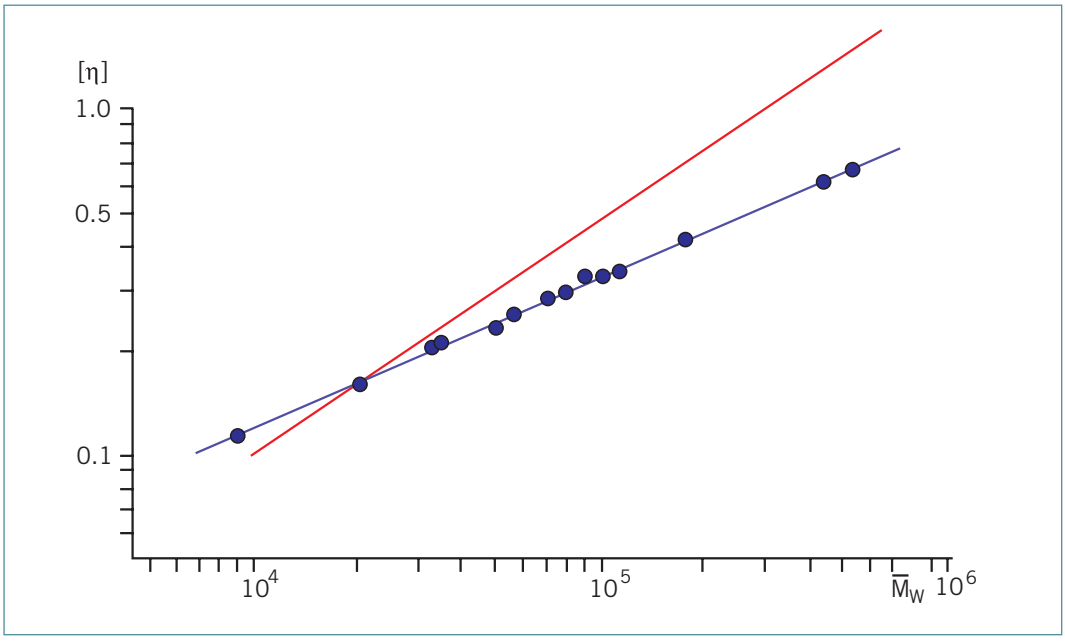



**Fig 3.** Log-log plot of [η] against $\overline{M}_w$ for dextran B-512(F) (blue line) and for a hypothetical linear dextran (red line) (47).

The deviation from linearity at higher MW is ascribed to the increase in branching and the polymolecularity of the fractions used. Granath (52) obtained the following relationship for dextran in the clinical range.

$$[\eta] = 2.23 \times 10^{-3}\ \bar{M}_W^{0.43} \quad \textit{(for MW <5 x 10}^5\textit{)}$$

Recently a value of 0.47 was derived for the exponent with dextran fractions of molecular weights up to 500 000 (57). The value of the exponent decreases with increasing MW and for a $\overline{M}_W$ of $>10^6$ a value of 0.22 has been derived (47). The Newtonian behaviour of dextran solutions is ascribed to their branching.

From measurements of g, which represents the ratio of the mean square radius of gyration of branched molecule to that of an unbranched molecule of the same $\overline{M}_W$, Bovey (46) and Senti *et al.* (47) calculated that, when the presence of a high percentage of single branches was taken into account, agreement could only be obtained by assuming that the long branches must be at least 50 units long. This compares well with the implications from Walker's enzyme studies. Wales *et al.*(56) applied similar theory to calculate average branch lengths and spacings for a dextran fraction, $\overline{M}_W$ approx. 300 000, and derived figures of 6 and 9 glucose units respectively. More recently, Garg and Stivala (58) derived g values from low angle X-ray scattering and obtained similar values for the branching. However, neither of these studies has taken into account the fact that at least 80% of the branches are <2 units long. There is some evidence that the longer chains have longer branches (57).

Many aspects of the fine structure of dextran remain to be resolved, in particular, whether the branches are part of a comb-like structure or a more ramified structure. The acceptor mechanism would in fact support the latter.

The colloid osmotic pressure of dextran solutions, a parameter of very considerable importance in its plasma volume expansion effects, has received much attention in our laboratories and elsewhere (60, 61). The concentration dependence is depicted for Dextran 40 and 70 in Figure 4. The increasing divergence of the osmotic pressures of dextran from those of albumin at higher concentrations reflects the greater interaction between dextran and water, which is a good solvent for dextran, and the properties conferred by the random coil structure (62).

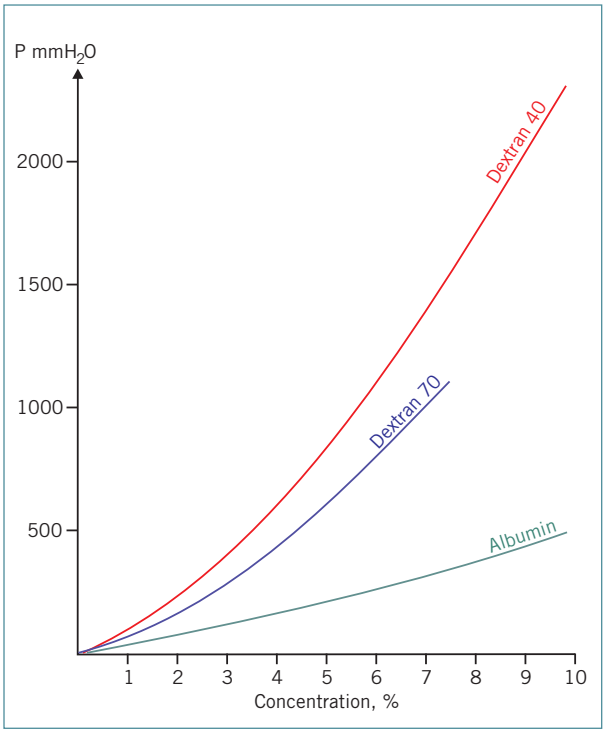



**Fig 4.** Plot of osmotic pressure (mm $H_2O$) against concentration (%) for Dextran 40, Dextran 70, and albumin.

Studies on dextran diffusion through a range of membranes suggest that dextran is less hindered than would be predicted on the basis of Stokes' radius (63, 64).

The α(1→6) linked polysaccharides represent a class of very flexible and extended polymers (65). Above $\overline{M}_W$ 2 000, dextran behaves in solution as an expandable coil. The molecular dimensions are dependent on the solvent, and the MW and concentration of the polysaccharide (see Table 3). The solution properties indicate that dextran molecules with MW >$10^5$ behave as if they were highly branched. As the MW increases further, the molecules attain even greater symmetry (47, 52, 56).

| $\overline{M}_W \times 10^{-3}$ | Radius of gyration Å | Stokes' radius Å |
|---|---|---|
| 2000 | 380 | 270 |
| 1000 | 275 | 199 |
| 500 | 200 | 147 |
| 200 | 130 | 95 |
| 100 | 95 | 69 |
| 70 | 80 | 58 |
| 50 | 68 | 49.5 |
| 40 | 62 | 44.5 |
| 10 | — | 23.6 |

**Table 3.** Molecular dimensions of dextran B512 as radius of gyration of Stokes' radius (52)

In the oligosaccharide range $\overline{M}_W$ <2000, the solution properties are best explained by a transition from a coil to a rodlike conformation (66). Dextran coils are rather compact in poor solvents, e.g. ethylene glycol, but they expand considerably in a good solvent such as methyl sulphoxide or formamide (55). Garg and Stivala (58) have shown by Small Angle X-ray Scattering (SAXS) that the molecular dimensions of dextran decrease with increasing concentration (0.5–3.0%).

Several attempts have been made to crystallize dextran in order to record X-ray diffraction patterns and thereby elucidate the detailed molecular architecture. Jeanes (67) obtained X-ray line patterns from samples of "crystalline" dextrans prepared by humidification or ethanol treatment. Barham (68) described an improved procedure for preparing spherulitic films from dextran fractions as reported earlier by Pasika and West (69). The spherulites gave similar powder diagrams to those obtained by Jeanes. Single crystals have been grown from a dextran fraction, $\overline{M}_W$ 19 900 (70–72). From the electron diffraction data, it was proposed that dextran adopts a ribbon-like conformation with consecutive glucose units in a near twofold screw relationship, the unit cell containing two antiparallel chains of two glucose units.

## 2.3. Reactivity

The reactivity of dextran involves primarily a study of the relative reactivities of the secondary, equatorially orientated hydroxyl groups, HO-2, HO-3, and HO-4. A small percentage of the hydroxyl groups in dextran are primary (approx. 1.5%), although this figure increases slightly at low molecular weights owing to the contribution from the non-reducing end-groups.

Attempts to tritylate the primary hydroxyls selectively in methyl sulphoxide/pyridine have been reported. Although Rees and coworkers (73) found that some tritylation of secondary hydroxyls could not be avoided, the reaction was used successfully by Larm (30) for studying the length of the branches.

Studies on the partial methylation (74) revealed the following relative reactivities k2:k3:k4 = 8:1:3.5. Good agreement between the experimental and theoretical distribution of methyl ethers was only obtained when allowance was made for the enhanced reactivity at HO-3 as a result of substitution at HO-2 or HO-4. Thus, for example, k3 is enhanced by a factor of 5.2 by substitution at both HO-2 and HO-4 (74).

As with other glucans, the reactivity at HO-2 towards alkylating agents is higher than at HO-3 or HO-4. This is rationalized in terms of the higher acidity of the HO-2 due to its proximity to the anomeric centre (75). An investigation of the partial methylation of some model compounds, for example, methyl α- and β-D glucopyranosides and their 6-O-substituted derivatives, indicated that no special effects need be invoked in the polymers to explain the low reactivity at HO-3 (76). When HO-2 and HO-4 are ionized, the reactivity at HO-3 is depressed. However, substitution at HO-2 or HO-4 abolishes this effect with subsequent increase in reactivity at HO-3 (74).
Some caution in applying these generalizations is needed, as the base strength may affect the relative and absolute reactivities of the hydroxyls (77, 78).

The relative reactivities of the hydroxyl groups towards ethylene oxide closely follows those for methylation (79). At higher degrees of substitution (DS), however, the pattern of substitution becomes complex owing to the introduction of primary hydroxyl sites.

Acylations may differ from alkylations in that they may be subject to thermodynamic control owing to migration of the substituents. A study of the partial acetylation of dextran with acetic anhydride/pyridine (80) revealed that k2>k3=k4. This order of reactivity is similar to that shown by methyl β-D-glucopyranoside. When, however, the acylation was conducted in aqueous alkali, the reactivities were virtually identical, indicating rearrangement.

An investigation of the distribution of sulfate groups in partially sulfated dextran, using dextran N-4 with a similar structure to B-512(F), showed that HO-2 was again highest with k2:k3:k4 = 1.6:1.06:1.0 (81). The percentage of di-substituted glucose units was surprisingly high even at low DS.

Carbonyl groups may be introduced into dextran by Fenton's reagent (82), methyl sulphoxide/acetic anhydride reagent (83), or by means of aqueous bromine at pH 7 (84). With the latter reagent, oxidation appears to occur mainly at C-2 (21%) and at C-4 (25 %). Similar patterns are obtained with methyl α-D-glucopyranoside (85). When the oxidation is performed in the presence of borate, less oxidative cleavage to dicarbonyl moieties results (86).

# 3. Biosynthesis

## 3.1. Dextransucrase (Sucrose: 1,6 α-D-glucan 6-α-glucosyl transferase, EC. 2.4.1.5.)

### 3.1.1. Purification

Although the slime-forming properties of filtered extracts of *Bacillus mesentericus* were first noted by Beijerinck (87) in 1910, the evidence published by Hehre (88, 89) in 1939 and 1941 provided a more rigorous proof of the dextran synthesizing activity present in *Leuconostoc* extracts. During the next two decades, a surge of activity led to a rapid expansion in our knowledge of the properties of dextransucrases. Although a number of refinements in the isolation of the enzyme were made during these years, the first attempts to purify the B-512(F) enzyme were described by Ebert and Schenk (90).

Their procedure entailed methanol precipitations, calcium phosphate chromatography and ammonium sulphate precipitation and gave high activities. Values of 2000 units per milligram (U/mg) residual dextran were reported. The purification of the enzyme was re-examined by Robyt and Walseth (91). Removal of the bound dextran by treatment with an endo-dextranase constituted an important and novel step in the purification. The enzyme concentrate displayed a specific activity of 53 U/mg (33% recovery) following the final ultrafiltration. Examination of the purified enzyme by PAGE revealed only two bands, both of which possessed dextransucrase activity. The faster band was assigned to the monomer and the slower to aggregates. On standing, the purified enzyme aggregates (91, 92). Levansucrase and invertase for example were absent from the preparation. The impure enzyme is not precipitated by ammonium sulphate even at 80% (w/v) (90). Itaya (93) reported that the enzyme could be precipitated directly from the culture by ammonium sulphate provided ovalbumin was added.

The existence of multiple forms of dextransucrase from B-512(F) was established by GPC and electrophoresis. A component giving a single band on PAGE and isoelectric focusing was isolated and identified as a dextransucrase. It yielded a dextran very similar to that obtained from a cell culture (92). The purified enzyme rapidly lost activity at 4 °C and even at -15 °C its activity decreased by 60% over 20 days. The enzyme may be stabilized, however, by addition of dextran but a minimum concentration of 4 mg/ml is required. The disparity between reports in the literature on the stability of dextransucrase preparations is, presumably, attributable to variations in the residual dextran concentrations. Lyophilized preparations of the purified enzyme appear to be stable indefinitely (92). For the purification of *Leuconostoc* dextransucrases, a preliminary treatment with dextranase may be followed by one of several techniques. Polyethyleneglycols ($\overline{M}_W$ from 400–6000) at concentrations from 5% to 40%, respectively, effectively precipitate the enzyme (94).
Gel permeation chromatography on Bio-gel A5m or Sepharose™ 6B (94–96) and ion-exchange chromatography on a DEAE-type gel (93–97) have frequently been used. Hydrophobic chromatography using O-(phenoxyacetyl)cellulose was found to be advantageous with the B-1355 dextransucrases (94). McCabe and colleagues (98) found that dextransucrase displayed an affinity for Sephadex™. This technique has also been applied *Leuconostoc* enzymes (99). The purified enzyme, however, does not shown any affinity for Sephadex™ G-100 (92).

### 3.1.2. Properties

The highly purified dextransucrase isolated by Robyt and Walseth (91) appears to be a glycoprotein with mannose as the preponderant sugar. Only trace amounts of glucose were detected.

The importance of calcium ions for the activity of the enzyme had already been noted by Brock Neely and Hallmark in 1961 (100). Robyt and Walseth (91) found that the activity of the enzyme which had been deactivated by incubation with EDTA could only be fully restored by calcium ions. They suggested that the enzyme was a calcium metalloprotein. Similar calcium dependence has been noted for dextransucrase from *Leuconostoc mesenteroides* IAM 1046 (93).

Studies on the nature of the catalytic site of the enzyme suggest that polyisophenol phosphates are involved (96, 101, 102).

The value (284 000) for the MW of the B-512(F) dextransucrase (90) is almost certainly too high. The preparations used may not have been entirely free from bound dextran and may also have been aggregated. In a recent study of the B-512(F) enzyme, a value for the monomeric enzyme of 64 000 by SDS gel electrophoresis was reported (92). The MW's reported for the Streptococcal enzymes were 94 000 for *S. mutans* 6715 (103) and 102 000 for *S. sanguis* 10558 (96) although for *S. mutans* HS-6, a value of 170 000 has been quoted (95).

The activity of the enzyme depends, *inter alia*, on pH. The optimal pH extends over a rather narrow range, 5.0-5.3. At this pH, the enzyme is also most stable and a partly purified sample was found to retain its activity for at least 24 hours at 2 °C (18, 104, 105).

### 3.1.3. Donor substrate specificity

Dextransucrase is in many respects a remarkably versatile enzyme. A wide range of products can be formed depending on the nature and concentrations of the donor and acceptor substrates.

It was believed for a long time that sucrose was the only natural substrate for dextran sucrase. However, during the past years, a number of other donors, both natural and synthetic, have been recognized. Thus Genghoff and Hehre (106) found that α-D-glucopyranosyl fluoride yields a high molecular weight dextran in the presence of dextransucrase from *Leuconostoc mesenteroides* B-512(F) or *Streptococcus DS var*. Indeed, the kinetic parameters for the reactions were virtually identical to those for sucrose (107). These findings are of particular interest as they indicate that the synthesis operates via glucosyl transfer in accordance with the generally agreed mechanism.

Lactulosucrose (O-α-D-galactopyranosyl-(1→4)-O-α-D-fructofuranosyl-(2→1)-α-D-glycopyronoside) is reported to give dextran when incubated with various Leuconostoc dextran sucrases (108).

Recently, Binder and Robyt (109) reported that p-nitrophenyl-α-D-glucopyranoside acted as a donor substrate for dextransucrase (purified preparation) form *Leuconostoc mesenteroides* B-512(F) and *Streptococcus mutans* 6715. The products were high MW dextrans and nitrophenyl isomaltodextrin glycosides. The rate to transfer was, however, very much less than that for sucrose.

Evidence has been adduced that C-3, C-4 and C-6 are important sites for the binding of sucrose to dextransucrase (110).

In 1960, Tsuchiya (13) observed that when dextran and dextransucrase were incubated in the absence of sucrose with suitable acceptors, for example glucose and maltose, the corresponding oligosaccharides were produced. He concluded that even dextran could act as a donor and this has recently been reaffirmed by Binder and coworkers (99). By means of a chromatographic study using purified enzymes, they found that panose, isomaltotriose and dextran ($\overline{M}_W$ approx. 10 000), which are all recognized as good acceptors, also acted as glucosyl donors, and, in the absence of sucrose, afforded oligosaccharides.

## 3.2. Mechanism

To establish the mechanism of action of dextransucrase, Robyt and coworkers (111) used an immobilized form of the enzyme for a series of "pulse and chase" experiments. The soluble dextransucrase was covalently bound to Bio-gel P2 beads by the ethylenediamine glutaraldehyde technique and then incubated with a low concentration of $^{14}$C-sucrose (pulse) and, thereafter, with a high concentration of cold sucrose (chase). Similar experiments were also performed with the *Leuconostoc* cells to which the enzyme is naturally bound.

After thoroughly washing the gel or cells, the products were released by treatment at pH 2 for 10 min at 95 °C. Only two products were found, namely dextran and glucose. It was established that the glucose was not due to hydrolysis of the dextran during the release operations. Following the pulse experiment, activity was located in both the glucose and the dextran but, after the pulse and chase, only in the dextran.

An essential part of the evidence was derived by reduction and hydrolysis of the dextran produced. The activity of the sorbitol and glucose produced was measured. After the pulse the ratio of activities for sorbitol/glucose was considerably higher than those after the pulse/chase experiment.

A mechanism for the biosynthesis was thus proposed in which the enzyme serves two functions: firstly, hydrolysing the sucrose and binding the glucosyl moiety, thereafter, building up the dextran chain by an insertion mechanism (see Figure 5).

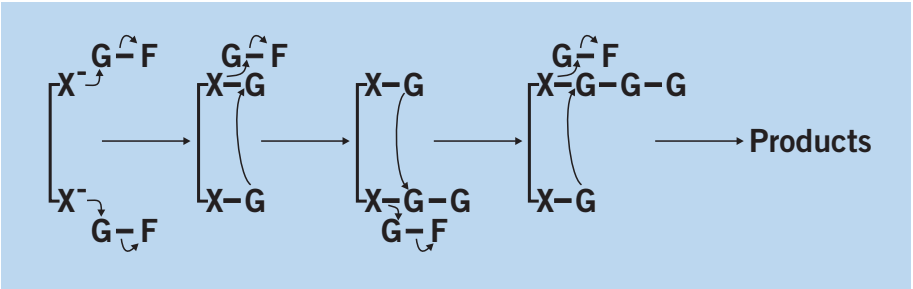



**Fig 5.** Two site mechanism for the biosynthesis of dextran chains by dextransucrase. G-F represents a sucrose molecule composed of glucose (G) and fructose (F) molecules. G-G represents α(1→6) linked glucose residues. The binding sites X on the enzyme may not be structurally similar (adapted from Robyt *et al.* [111]).

The mechanism may be summarized as follows:

1. Formation of the glucosyl derivatives on the enzyme.
2. Attack by the primary hydroxyl of one glycosyl moiety on the anomeric carbon of the other glucosyl moiety to form a disaccharide.
3. Formation of new glucosyl derivative at the unoccupied site.
4. Attack by the primary hydroxyl of the latter on the anomeric carbon of the disaccharide to form a trisaccharide.
5. Repetition of these processes.
6. Termination reaction.

This mechanism has been simulated with molecular models (101). Although certain criticism has been directed at the experimental work of Robyt and coworkers, in particular, the purity of the enzyme preparation, the low activity of the bound enzymes and the prolonged incubation time with $^{14}$C-sucrose, the earlier conclusions were nevertheless vindicated. Ditson and Mayer (112), using a highly purified dextransucrase from *Streptococcus sanguis* ATCC-10558, were able to confirm that the glucose was incorporated at the reducing end of the growing chain. With their coupling technique, they were able to retain more than 80% of the enzymatic activity and pulse sequences of 10–30 s were sufficient to effect considerable polymerization. They showed that the labelled end groups incorporated during the short pulse phase declined abruptly during the chase with cold sucrose. More elaborate pulse and chase experiments have been performed with two purified enzymes from *Streptococcus mutans*. The results were again consistent with the insertion mechanism (113).

These studies (111–113) have amply confirmed the earlier proposals of Ebert and Patat (114, 115) in which the "insertion" type mechanism was first conceived.

The glucosylated form of the enzyme has been prepared and characterized by Mayer and colleagues (116, 117).

## 3.3. Acceptor reactions

A number of sugars and sugar derivatives function as glucosyl acceptors, in particular maltose, isomaltose, methyl α-D-glucoside and low molecular weight dextran (118, 119). Experiments with $^{14}$C-glucose, $^{14}$C-fructose and $^{14}$C-end-labelled maltose in the presence and the absence of sucrose (120) confirmed the hypothesis that dextransucrase covalently binds glucosyl and dextranosyl groups and that these are released from the enzyme by the acceptor by attack at the reducing end. This mechnism is consistent with the following observations;

(1) the acceptor reaction occurs in the absence of sucrose;
(2) the acceptor molecule is incorporated at the reducing end of the product;
(3) the products are dextrans and low molecular weight oligosaccharide(s);
(4) as the ratio of acceptor to sucrose increases, the proportion of dextran decreases and that of oligosaccharide increases.

Homologous series of oligosaccharides can be accounted for by assuming that as each member is formed it acts as a new acceptor, thereby giving rise to the next higher member of the series.

A more detailed study of the acceptor activity of various sugars was undertaken by Mayer and coworkers (121). They studied the time course by the glucosyl transfer to these acceptors, and found that the appearance of the homologues was consistent with the sequential transfer of glucosyl units to the acceptor.

Robyt and Eklund (101) listed 26 known acceptors of varying activity and recently explored the quantitative aspects of acceptor activity in the dextransucrase system. The products were dextran and in certain cases a homologous series of isomalto-oligosaccharides. Glucose, being the product of the acceptor reaction of water, was also found in small amounts. Two acceptor products from fructose were always present in small proportions; leucrose (O-α-D-glucopyranosyl-(1→5)-D-glucopyranse) approx. 2% and isomaltulose (O-α-D-glucopyranosyl-(1→6)-fructofuranose) approx. 1%. The products of an acceptor reaction will, however, depend, *inter alia*, on the ratio of acceptor to substrate (122).

Walker's findings (123) can now be rationalized in terms of an acceptor mechanism. She found that when $^{14}$C-sucrose and an excess of isomalto-oligosaccharides were incubated with dextransucrase, the main product was the next higher homologue with the radioactive label attached to the non-reducing end.

However, the issue has recently become more complicated by the observations that dextran and a number of D-glucosyl oligosaccharides (e.g. isomaltotriose, panose) may in fact serve both as glycosyl donors and acceptors for dextransucrase (99). Interestingly, they also established that the enzyme could transfer single glycosyl units from dextran to an acceptor molecule.

More recently, Luzio and Mayer (124) have stressed the dependence of the acceptor reaction on concentration. They reported that at low sucrose concentrations, 0.05 mM to 0.5 mM, the acceptor reaction of water became significant and the hydrolysis of the sucrose and fructose preponderated over dextran synthesis.

The fact that hydrolysed dextran serves as an efficient acceptor had already been established in the 50's (125, 126). Hehre (125) found that a supplement of 1 mg/ml of low MW dextran markedly lowered the MW of the dextran produced by dextransucrase. This finding was developed by Hellman and colleagues (126) at Peoria who used a 2% supplement of dextran, $\overline{M}_W$ 17 600, and obtained a 33 % yield of a dextran in the clinical range. They also found that the yields increased as the MW of the added dextran decreased.

Using $^{14}$C-labelled dextran, Mayer and his colleagues (121) found that the MW of the acceptor dextran increased significantly during the incubation with dextransucrase, thereby confirming its role as an acceptor. Furthermore, the kinetic data identified hydrolyzed dextran ($\overline{M}_W$ approx. 10 000) as one of the most potent acceptors with a Km lower than that of maltose and isomaltose and V 4–5 times higher than any other sugar. Their results thus corroborated the earlier findings of Robyt and Corrigan (127). The addition of dextran ($\overline{M}_W$ approx. 70 000) to a purified, dextran-free enzyme caused a 5-fold increase in the activity (129). From kinetic data, it was concluded that the binding of dextran to the enzyme also increases the affinity of the enzyme for sucrose.

## 3.4. Branching

Attempts to rationalize the branching activities of dextransucrase are still hampered by an incomplete knowledge of branching in native dextran. Ebert and his colleagues (15, 115, 128) had originally proposed a branching mechanism based on an acceptor reaction whereby growing dextran chains (or oligosaccharide chains) are transferred consecutively to the acceptor dextran by the enzyme, thereby giving rise to a branched dextran. Their hypothesis was based on a study of the products obtained by adding a radioactive-labelled acceptor dextran of $\overline{M}_W$ approx. 25 000, to purified enzyme and sucrose (5%). Enzymes from B-512(F), B-1299 and B-1307 were studied. The MWD of the products was examined (although not quantitatively) and the specific activity was also investigated. The results clearly demonstrated that the dextran produced contained one acceptor molecule. However the nature of the new linkage was not established.

More compelling evidence for the acceptor mechanism of branching was accrued by Robyt and Taniguchi (130) who, using radioactive-labelling, established the presence of newly formed α(1→3) linkages. The pattern of labelling was consistent with the aforementioned acceptor mechanism. To account for the presence of branches in a native dextran, synthesized by enzyme and sucrose only, it is presumed that free dextran molecules are released into the media by acceptor reactions with glucose or fructose. The branching mechanism, which is essentially an acceptor reaction, is depicted in Figure 6.

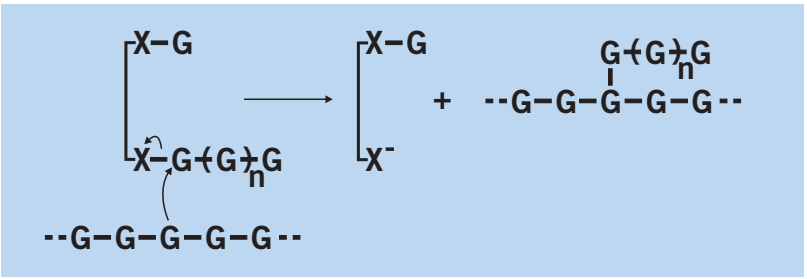



**Fig 6.** Mechanism for the formation of branches in dextran B-512(F). The sequence --G-G-- represents part of a dextran chain (adapted from Robyt *et al.*[111]).

All attempts to demonstrate the presence of a separate branching enzyme have so far failed. The presence of strongly bound dextran on the enzyme complicates the purification. Recently, the degradation of this bound dextran by an endodextranase has been made possible. In this way, Côté and Robyt (94) were able to separate two extra-cellular enzymes from *Leuconostoc mesenteroides* B-1355, one synthesizing alternan, a glucan with alternating α(1→3) and α(1→6) linkages and the other synthesizing a dextran similar to B-512(F).

Recently, however, Kobahashi and coworkers (92, 129) demonstrated that a purified dextransucrase from B-1416 gave only a single protein band with dextran-synthesizing activity on PAGE. They also isolated an apparently homogenous protein from B-512(F) dextransucrase which appeared as a single band on PAGE and isoelectric focussing. This enzyme produced a dextran very similar to the dextrans produced by standard enzyme preparations. To account for the fact that highly branched dextrans, such as B-742, have, preponderantly single branches, whereas B-512(F) has a low proportion of longer branches, Côté and Robyt (131) postulated that the greater affinity between the acceptor and the binding site on the enzyme and the resulting increased rate of the acceptor reaction relative to the chain elongation reaction will promote shorter branches.

# 4. Production of clinical and technical dextran

Dextran for clinical and technical products is produced in most developed countries throughout the world. Reliable figures for the annual world production of dextran are not available but an estimate based on the units of clinical dextran consumed in some countries would lead to a figure in excess of 500 metric tons.

In the West, most producers use the *Leuconostoc mesenteroides* NRRL B-512(F) or B-512 strain for the fermentation. In other parts of the world, alternative strains appear to be used (132, 133).

Most major producers of dextran employ a process based on the batchwise culture of *Leuconostoc* in the presence of sucrose. The viscous culture fluid is then precipitated in ethanol or methanol, whereafter the native dextran obtained is hydrolyzed in dilute acid and the desired dextran is isolated by fractionation. Although the present state of the art offers alternative methods of producing defined fractions, most producers are still operating a procedure introduced about 35 years ago. In introducing any change, a producer must be convinced that, not only must the new process be more efficient in man-power and materials, but the final product must conform in every respect with the medical requirements for safety and efficacy.

The organism, *Leuconostoc mesenteroides* NRRL B-512(F), is a member of the Lactobacillaceae family, genus *Leuconostoc* and species *mesenteroides* (134). The organism produces spherical or ovoid cells and classifies as a gram-positive facultative anaerobe. Apart from dextran and lactic acid, it produces, *inter alia*, carbon dioxide, ethanol, mannitol and acetic acid.

## 4.1. Fermentation

Although many sugars, for example, glucose invert sugar and maltose, will serve as energy sources for the growth of the bacteria, only sucrose serves to induce dextransucrase production (89, 104, 135–137). Thus the culture media are necessarily based on sucrose supplemented with various nutritional requirements. Nicotinic acid, thiamine, pantothenic acid have been found essential for all *Leuconostoc* and cystine, glutamic acid, isoleucine and valine for *Leuconostoc mesenteroides* in particular (138–141). In practice, these requirements are satisfied by yeast extracts, corn steep liquors, acid hydrolyzed casein or malt extracts generally with the addition of peptone or tryptone broth (104, 142–145). Jeanes (142) has described the laboratory production of dextran in excellent detail. The dextrans obtained from cultures using yeast extract, malt extract or liver extract respectively, were similar (145). It is worthy of note that even at concentrations of nutrients one quarter of those generally employed in the fermentations, the yields of dextran were unchanged although longer times were required (146).

In addition to the presence of various cations, phosphate salts (e.g. sodium dihydrogen phosphate or hydrogen phosphate) at 0.5% are added. For 2% corn steep liquors, Tsuchiya and coworkers (104) found 3.5% potassium dihydrogen phosphate optimal. However, biomass production at 0.1% and 1% phosphate were found to be identical. Furthermore the decay in dextransucrase activity between 12 and 24 hours was much less with 0.1% phosphate (147).

In the laboratory, shaken cultures are claimed to give consistently higher yields than still cultures (104).

## 4.2. Elaboration of dextransucrase

The factors influencing the elaboration of the enzyme have provided a fertile area of study.

### 4.2.1. pH

The pH affects the production of enzyme by the organism, the activity of the enzyme and its stability. Unfortunately, these optima are not identical. Initial pH values for the fermentation media generally lie between 6.7 and 7.2. Tsuchiya (104) noted that vigorous production of the enzyme began after approx. 6 hours when the pH had fallen from 6.9 to 6.4 (see Figure 7).

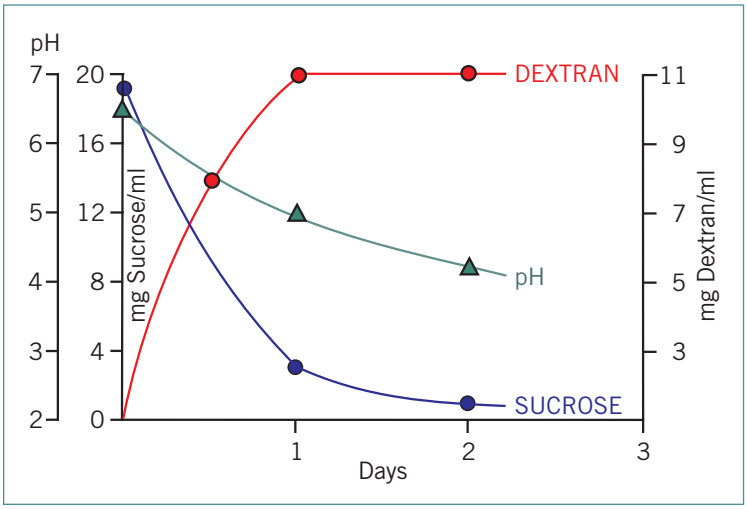



**Fig 7.** Changes in the sucrose concentration, pH, and dextran concentration during the fermentation of *Leuconostoc mesenteroides* (137).

They further showed that the maximal enzyme activity (as measured by reducing sugar liberation under standard incubation conditions) was obtained in cultures maintained at pH 6.7.

Tsuchiya and colleagues (119) recommended the addition of alkali to the culture to maintain a pH of 6.7 until maximal enzyme activity is attained, whereafter the pH is allowed to fall to approx. 5, where the enzyme acquires its maximal activity and stability (see section 4.3.1).

### 4.2.2. Sucrose concentration

It was established at an early stage that, for sucrose concentrations from 0.5% to 8.0%, increasing sucrose levels afforded increasing dextransucrase activities (104, 136). The increase in dextransucrase activity is most marked at sucrose concentrations up to 2%; thereafter the increments are correspondingly less. Alsop (18), however, was not able to confirm a correlation between sucrose levels and dextransucrase activities. Lopez and Monsan (148) found that continuous feeding of sucrose to the culture led to greatly increased yields of dextransucrase. However, in selecting the optimal concentration of sucrose, the yield and quality of the dextran produced must also be taken into consideration (see section 4.3).

### 4.2.3. Time

Maximum yields of dextransucrase are recorded approx. 6-8 hours after the start of the fermentation (104, 105, 147, 148). Thereafter, the instability of the enzyme of pH >6.2 will contribute to a decline in activity.

## 4.3. Biosynthesis of dextran

### 4.3.1. pH

The dextran synthesizing activity of the enzyme exhibits a maximum at pH 5.2, which is considerably lower than that for the optimal elaboration of the enzyme in culture (104, 119, 149–151). It is, perhaps, fortuitous that the enzyme also exhibits maximum stability within the pH range 5–6.5 (105, 119, 149, 151-153). The stability of the enzyme is, however, dependent on the presence of other substances, of which high molecular dextran acts as a stabilizer *par excellence* (154).

As the fermentation proceeds, the pH falls from the initial value, 7.2, to approx. 5 within 20 hours owing to the liberation of organic acids, thereby, creating an environment favourable for the synthesis of dextran. Although the periodic neutralization (pH 6.7) of the culture with 5–10 N sodium hydroxide in order to increase dextransucrase levels has been recommended, there is little evidence that this also leads to improved yields of high quality dextran (104, 135, 143, 155). High pH (8) and low temperature (20 °C) appear to favour the formation of levan (3).

### 4.3.2. Sucrose concentration

Very little dextran is produced in cell cultures at sucrose concentrations under 2%; higher concentrations may significantly influence the yield, the molecular weight distribution, and the structure of the dextran. Hehre (144) demonstrated that increasing concentration (0.5–5%) of sucrose afforded increased yields of dextran. Detailed studies on the effects of higher concentration (10–50%), albeit mostly in cell-free systems, have revealed that the yields of high MW dextran decrease accordingly with corresponding increases in the proportions of low molecular dextran (18, 119, 126, 149). The yields of dextran ($\overline{M}_W$ <5000) obtained at various sucrose concentrations are shown in Table 4. The figures for the highest sucrose concentrations are derived from enzyme studies as the cells do not flourish at sucrose concentrations >20-25%.

| Sucrose concentration (%) | Yield of dextran % (g/100 g sucrose) | |
|---|---|---|
| | ref. 18 | ref. 107 |
| 2 | 45.9 | — |
| 5 | 44.4 | — |
| 10 | 39 | 38 |
| 20 | 17.9 | — |
| 30 | — | 17 |
| 50 | — | 2.5 |

**Table 4.** Effect of sucrose concentration on dextran yield.

The remaining material consists of mono- and oligo-saccharides and low MW dextran. Precise data on the molecular weight distributions of these fractions is lacking. A further complicating factor is the observation that the branching ratio decreases with increasing sucrose concentration. Thus, at a sucrose concentration of 30%, the ratio of (1→6) links to non-(1→6) links was 29 whereas at 5% sucrose, the value was 8.5 (156). These values were obtained by periodate oxidation and have not been confirmed by modern techniques.

### 4.3.3. Temperature

Published studies using dextransucrase concentrates, assuming that they can be extrapolated to whole cell conditions, suggest that temperature may influence the process in many ways: (a) yield of high MW dextran; (b) rate of formation; (c) MWD and structure of dextran formed. The yield of high MW dextran decreases at lower temperatures (range 25 °C to 4 °C) with a concomitant increase in the low MW fraction (119, 126). Interestingly, the overall yield did not appear to change (see Table 5).

| Temp. °C | Total dextran % | Low MW % | High MW % |
|---|---|---|---|
| 30 | 45.9 | 21.2 | 24.7 |
| 15 | 47.4 | 43.4 | 4.0 |
| 4 | 47.2 | 45.9 | 1.3 |

**Table 5.** Effect of temperature on the MWD of dextran (126).
% is based on added sucrose. Yields of low and high MW dextran were determined by fractional ethanol precipitations.

At temperatures exceeding 35 °C, Hehre and Sugg (152) found that the yield of dextran was considerably less than at 23 °C. Above 32 °C, the enzyme is unstable (149, 151-153).

Kinetic studies (149, 157) using an enzyme concentrate showed that the rate ($V_{max}$) increased 10-fold between 0 °C and 32 °C for sucrose concentrations from 2 to 6%. Tsuchiya and coworkers (119) noted that the time required for complete conversion at 15 °C was double that at 30 °C.
For practical purpose, a temperature of approx. 25 °C is recommended. At this temperature the dextran can be recovered after 24–28 hours (135, 142, 143, 158). Studies based on light-scattering, viscosity and enzymes have established that the branching increases with synthesis temperature in the interval 0–30 °C (159, 160).

### 4.3.4. Calcium

The dependence of dextransucrase activity on calcium was first noted by Brock Neely and Hallmark (100), albeit for the enzyme from *Betacoccus arabinosaceous*. Later, the calcium dependence of dextransucrase (*Leuconostoc mesenteroides* IAM 1046) was confirmed but dextran yields were not improved in the presence of excess calcium (93). Jeanes (135) had earlier reported that neither yield nor viscosity of the dextran seemed to be improved by including 2% calcium carbonate in the culture medium.

Robyt and Walseth (91) reported a twofold increase in enzyme levels in whole cell cultures in the presence of 0.001–0.1% calcium chloride (see Figure 8). Lopes and Monsan (148) found that dextransucrase activities elaborated at 0.005 and 0.05% calcium chloride were similar. In considering the effects of calcium, it is important to note that the solubility of calcium hydrogenphosphate in water is only 31 mg/100 ml. In practice, much calcium may be filtered off after the addition of phosphate.

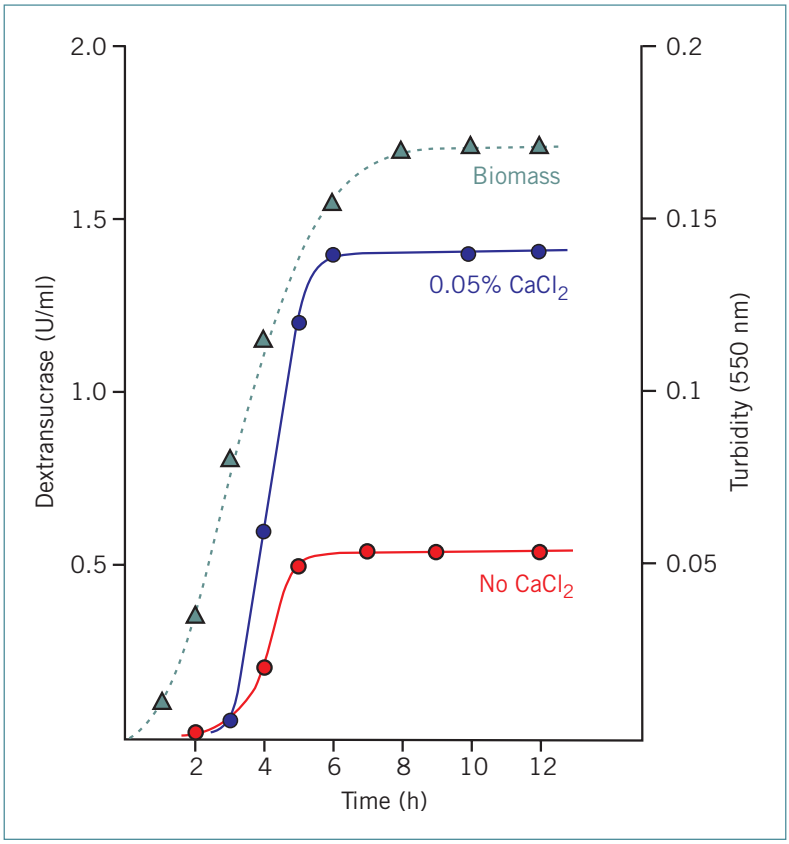



**Fig 8.** The production of extracellular dextransucrase in the absence of calcium chloride and in the presence of 0.05% calcium chloride. Biomass was similar in both cases (adapted from Robyt and Walseth [91]).

### 4.3.5. Time

Unlike several other strains of *Leuconostoc*, prolongation of B-512(F) cultures beyond 24 hours leads to a decrease in the molecular weight of the isolated native dextran (135, 158). When a fermentation was permitted to continue for 273 hours, the resulting dextran had a $\overline{M}_W$ of 8.6 millions compared with 39 millions after 23 hours. The total yield of dextran was however unchanged (158). In whole cell cultures, a significant decrease in the relative viscosity after approx. 50 hours fermentation was found, at which time the yield had attained its maximum (143). Braswell and coworkers (156, 161) did not detect any decrease in MW (light-scattering) during the enzymatic synthesis but the time scale of their experiments is uncertain. Likewise, Tsuchiya and colleagues (119) reported little change in MW of the dextran elaborated by an enzyme concentrate.

## 4.4. Clinical fractions

The conversion of native dextran to refined clinical fractions embraces two key operations.

1. The partial hydrolysis of native dextran to products containing appropriate molecular sizes.
2. Fractionation by means of ethanol or methanol to give clinical dextrans.

The fractions most commonly used in medicine have $\overline{M}_W$ of approximately 70 000 or 40 000. In the official monographs, the molecular size specifications are stipulated in terms of the limits for the average values for the total distribution and of the 10% high and 10% low methanol precipitated fractions. These values are expressed in terms of either the $\overline{M}_W$ or [η]. For an authoritative review see Nilsson and Söderlund (162). Thus the prime aim of the manufacturer is to achieve maximal yields of dextran meeting these specifications as regards both MWD and purity.

### 4.4.1. Partial acid hydrolysis

Following the pioneering studies of Ingelman and Halling (163) on the hydrolysis and fractionation of dextran, extensive and detailed studies on the parameters influencing the hydrolysis of native dextran B-512(F) and the fractionation of the products appeared in 1954-5 (164, 165). It was found that only marginal improvements in the maximal yields of clinical dextran fractions could be achieved by varying the nature and concentration of the acid, the time and temperature for the hydrolysis and the nature of the organic solvent used for fractionation. Despite the advent of powerful modern techniques for studying distributions, few new studies have appeared.

Basedow *et al.* (166), studying kinetic aspects of the hydrolysis of narrow fractions ($\overline{M}_W$ 738 000, 72 700, and 4 380) concluded that the rate constant was dependent on the location of the bond in the chain, the bonds at the termini being more reactive to acid hydrolysis than those at the centre. This postulate was confirmed by product analysis using GPC (167).

Jones and coworkers (168) found that the rate constants for isomaltose and dextran are 12.3 $h^{-1}$ and 3.9 $h^{-1}$, respectively, implying that the MW indeed influenced the rate of hydrolysis. The rate constant for dextran corresponded to a degree of hydrolysis of less than 2% (based on bonds cleaved/total bonds). When considering the hydrolysis of native dextrans, particularly ones that are only slightly branched, the contribution of these effects to the product distribution is presumably minimal in view of the large size of the molecules and the fact that we are dealing with a degree of hydrolysis of 1.5% (bonds cleaved/total bonds). The hydrolysis conditions are thus chosen primarily with regard to operational factors and economics.

### 4.4.2. Fractionation procedures

After hydrolysis, the desired clinical fraction is isolated by fractional precipitations. Wolff and colleagues (164) found that a clinical fraction could be precipitated between 39–46% ethanol; for methanol slightly higher limits must be used (42-50%). The fractionation requires careful temperature control if good reproducibility is to be obtained. Using the same fractionation conditions, the yield and $\overline{M}_n$ of the fractions obtained at 20°C were 20% and 32 400, respectively, and at 30 °C, 33% and 47 600, respectively (164). The lower yield at 20 °C is due to the fact that more dextran appears in the non-clinical fractions at the lower temperature. The sedimentation of dextran on

fractionation has recently been interpreted in terms of mathematical models (169). The dependence of the solubility of dextran in ethanol/water mixtures on the MW is shown in Figure 9 (55). To aid coagulation and settling of the dextran precipitate, sodium chloride (2% based on the dextran present) should be added.

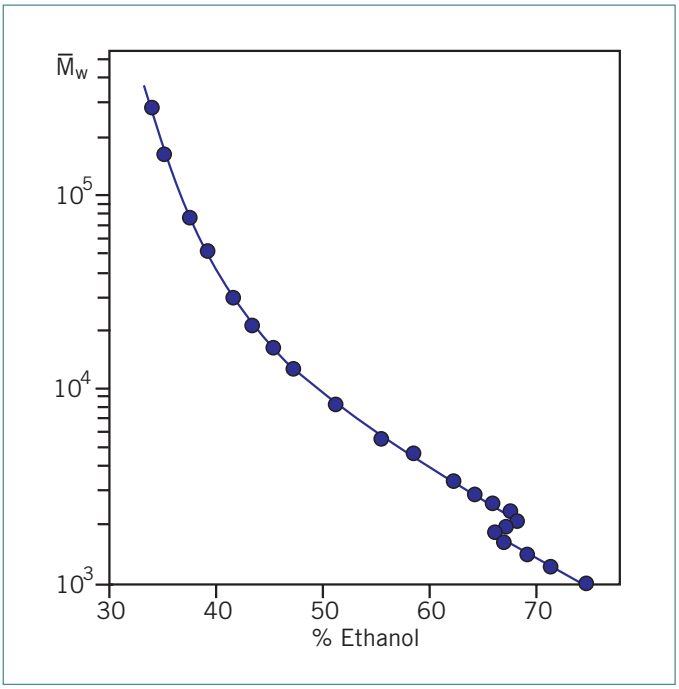



**Fig 9.** The solubility of dextran in ethanol/water mixtures at 25 °C. The $\bar{M}_w$ axis refers to the upper solubility limits for dextran (adapted from Basedow and Ebert [55]).

## 4.5. Quality assurance

Although clinical dextrans are submitted to many tests for chemical and biological (including immunological) purity, the MWD is recognized as one of the critical properties governing the duration of the fraction and its biological effects. The development of Gel Permeation Chromatography (GPC), pioneered for dextran control by Granath (170–173), provides a rapid and quantitative determination of the distribution. Further studies on GPC for measuring the dextran distributions and a critical analysis of the systematic and random errors involved has been presented by Nilsson and Nilsson (174). Alsop (175, 176) and Nilsson (177) have assessed the value of GPC in quality control of clinical dextrans.

In the monographs for Dextran 40, 60 and 70 which have recently been included in Ph.Eur(3rd ed.) and USP24 (only Dextran 40 and 70), a GPC method now replaces the outdated precipitation method for controlling the distribution.

In addition to the aforementioned controls, other routine tests for the acceptance of infusion solutions, for example pyrogens, particles, colour and sterility, must be performed. Over the years, however, studies on possible impurities have continued.

In studies aimed at detecting contaminating macromolecules, Richter (178) was unable to raise PCA reactive antibodies with the centrifuged supernatant of a crude hydrolyzate of native *Leuconostoc mesenteroides* B-512(F) dextran or a purified clinical dextran ($\overline{M}_W$ 70 000) in either rabbits or guinea-pigs and concluded that immunogenic impurities were absent. However, further studies based on reversed single radial immunodiffusion (RSRI) using high-titre antisera against the cellular (dextran-free) component of *Leuconostoc mesenteroides* revealed the presence of antigens in clinical samples from several countries (179). Some manufacturers have since then successfully eliminated these impurities from their products. The finding that the antigen cross-reacts with yeast mannan indicated that the antigen is, presumably, a mannano-peptide (179). Hedin (180) has examined the role of biologically active contaminants in clinical and other dextrans from the B-512(F) strain by means of the rat anaphylatoxin bioassay, the *in vitro* Limulus test and the rabbit pyrogen assay. Whereas these sensitive tests gave positive reactions in technical grade dextrans, the clinical products (Macrodex and Rheomacrodex) did not respond.

## 4.6. Stability in solution

Studies on the chemical stability of clinical dextrans at pH 4.5 to 7, when stored for several years at temperatures from 4 °C to 40 °C, revealed that dextran has excellent stability (181, 182). Even when severely handled with, for example, up to 50 freeze/thaw cycles, no significant changes in MWD, pH and buffer capacity were noted (183) (see Table 6).

| Treatment | $\overline{M}_W$ | $\overline{M}_n$ | $\overline{M}_W/\overline{M}_n$ |
|---|---|---|---|
| Reference sample | 69 500 | 41 500 | 1.67 |
| Freeze/thaw | 67 400 | 40 300 | 1.67 |

**Table 6.** Effect of freeze/thaw cycles on dextran

Lafrenz (184) investigated the stability of commercial dextrans in various glass and plastic flasks and found that changes in [η] were insignificant over 1 year (20 °C and 40 °C).

Soon after the large scale production of clinical dextran got under way, it became evident that flakes tended to form in a small proportion of the bottles. The flake formation was aggravated by freeze/heating cycles but only a few milligrams of the total amount present appeared fo form flakes. The flakes generally redissolved on heating (185, 186). Studies have confirmed that the flakes have a similar MWD to the soluble dextran in the bottle.

The tendency for orderly molecular association in dextran has been demonstrated by Jeanes (67) using X-ray diffraction patterns. Crystallinity was enhanced in hydrolysed dextran fractions particulary after treatment with 60–70% ethanol. Further evidence for time dependent intermolecular association in a Dextran 40 fraction was adduced by Aizawa and coworkers (187) using $^1$H-NMR, viscosity and IR measurements, albeit using rather high concentrations 10–50%.

## 4.7. Future developments

Native dextran is generally produced batch-wise in cell cultures. However, a number of alternative processes have been devised, for example (1) the use of cell-free enzyme extracts; (2) fermentations in the presence of acceptors; (3) continuous processes.

It was discovered some 30 years ago by Hehre (125) and Tsuchiya (188) that a supplement (1 mg/ml) of low MW dextran to the culture fluid markedly affected the MW of the dextran produced. This discovery was further exploited by Tsuchiya and coworkers (119) and Nadel (146). A 33% yield of clinical dextran has been obtained from an enzyme concentrate (126).

Few manufacturers seem to have adopted this technique. The reasons are many; (1) the main clinical fraction invariably appears to be accompanied by a very high MW fraction; (2) the yields may not exceed those obtained by standard procedures; (3) a separate process for the production of the low MW dextran must be devised; (4) new safety data to support the change-over must be assembled.

The production of dextran by immobilized dextransucrase has given promising results on a laboratory scale (148, 151, 155, 189). A clinical dextran has been produced by this technique (155). Studies to improve the existing methods of depolymerization and fractionation have continued.
The reports by Basedow and Ebert and colleagues (166, 190–192) on the effects of shear stress or ultrasound, either alone or in combination with acid hydrolysis, are of particular interest in this respect. These authors have also examined the products formed by the action of an endo-dextranase (193). There are indications that these procedures give rise to a less polydispersed product.

Conventional ethanol fractionation techniques are cumbersome and time-consuming. Production scale ultrafiltration has been available for many years. Unfortunately, this technique also has its limitations; perhaps the most serious is that dextrans exhibit poor selectivity with these membranes on account of their ability to deform within the pores of the membrane (194). Recently, however, Alsop and coworkers (195) have described a process involving GPC, ultrafiltration and ion exchange for the efficient fractionation of a dextran hydrolysate.

# 5. History of medical applications

## 5.1. Dextran 70

In the early 1940's, at the same time as Stacey and his associates (196, 197) in Birmingham were studying bacterial dextrans and Hehre and colleagues (89, 152) in the USA were pursuing the dextran producing activity of cell-free extracts of *Leuconostoc*, a young Swedish biochemist, B. Ingelman, at the Department of Biochemistry and Physical Chemistry, University of Uppsala began probing the polysaccharides and proteins of sugar beet juice.

One of the critical episodes was the discovery of dextran in an infected sample of the juice. This initiated a series of investigations on the polysaccharide (198). At the end of 1942, a recently qualified M.D., A. Grönwall, joined the laboratory to study tuberculin. Considerable effort was being devoted at the time to the freeze-drying of blood plasma for military medicine. Within the space of months, Ingelman and Grönwall had stumbled on the idea of using a hydrolyzed dextran as a plasma substitute. After studies on the partial hydrolysis, fractionation, and extensive biological studies, a Swedish pharmaceutical company adopted the project in 1943, and later that year, preliminary clinical trials began. In 1944, under the direction of the surgeon, G. Bohmansson, extensive clinical trials were started at the Regional Hospital in Örebro. The dextran used at that time was derived from *Leuconostoc mesenteroides*, strain 7E, and was slightly more branched than the present one. By 1947, about four years after the innovation, a 6% solution of a dextran fraction had been approved for clinical use in Sweden and, shortly thereafter, in the U.K., an achievement that would be inconceivable under the present regulatory climate.

The product was gradually improved and was designated Dextran 70. Samples of the Swedish product were soon tested clinically in the USA. Meanwhile, at the U.S. Department of Agriculture, Northern Regional Research Laboratory at Peoria, Allene Jeanes had been conducting studies on dextrans which were to have an important influence on the course of events (199, 200). During these studies, it had been established that a strain of *Leuconostoc mesenteroides* isolated from an infected bottle of root beer by R.G. Benedict and designated NRRL B-512 was a vigorous dextran producer and its dextran was only slightly branched. In 1949–50, strong interest among U.S. government and medical authorities and advocacy by Drs. Jeanes, Tsuchiya, and Koepsell at Peoria, stimulated a multi-disciplinary research program at the NRRC to provide basic information on the production of a synthetic blood volume expander from dextran. Progress was rapid and by the end of 1951, four American companies were producing dextran from the NRRL B-512(F) substrain for clinical purposes.

Numerous other dextrans had been compared but B-512(F) was deemed superior clinically and technically as a plasma volume expander. This substrain had been isolated from the original B-512 strain in 1948 by W.C. Haynes by selecting colonies with vigorous growth characteristics.
The activities of these colonies were followed through repeated growth cycles and the best results were obtained from a growth in a Fernbach flask, which thus gave rise to the designation (F) (199).
The production of clinical dextrans has since grown steadily throughout the world. Dextran 70 is generally marketed as a 6% solution in normal saline and as such continues to maintain its position worldwide as the plasma volume expander of choice. It is recommended for the treatment of shock or impending shock due, for example, to hemorrhage, burns, surgery or trauma (201–203).
Dextran 70 also reduces the risk for thrombosis and numerous studies testify to its value in significantly reducing the risk of post-operative fatal pulmonary emboli (204, 205).

## 5.2. Dextran 40

The introduction in 1961 of a further dextran product, Dextran 40, following a suggestion by Ingelman and Gelin (206, 207), was a direct consequence of earlier observations by Hint and Thorsen (208) on the erythrocyte disaggregating properties of dextran of $\overline{M}_W$ <50 000 and the classic report by Gelin on the circulatory disturbances following trauma and shock (209). Interest in this product was spurred by the important finding that low MW fractions also impart blood flow improvement properties by reduction of blood viscosity and inhibition of erythrocyte aggregation (210, 211). Although the optimal disaggregating effect was observed around $\overline{M}_W$ 25 000, a Dextran 40 was finally chosen with due consideration to renal excretion.

The antithrombotic effect of both Dextran 40 and 70, demonstrated experimentally by Gelin and colleagues (212) and clinically by Koekkenberg (213), provides a prophylactic treatment for deep venous thrombosis and post-operative fatal pulmonary emboli. A dosage not exceeding 20 ml/kg body-weight of dextran 40 (10% in normal saline) is recommended during the first 24 hours in patients undergoing high risk surgery or suffering from high risk trauma

## 5.3. Dextran 1

With the increased use of clinical dextrans, there followed an increase in the number of reports of dextran-induced anaphylactoid reactions (DIARs). The reported incidence from numerous studies varies from 0.03–4.7% (214). As these reactions are sometimes, albeit rarely, life-threatening, a collaborative study was conducted between 1968 and 1981 to elucidate the mechanisms underlying DIARs. Since severe reactions were shown to be antibody mediated, the idea of applying the hapten inhibition principle was examined. Following extensive animal and clinical trials, a monovalent hapten dextran fraction ($\overline{M}_W$ 1 000) was introduced in 1982. A small volume (20 ml of a 15% solution) was administered prior to the Dextran 40 or 70 infusion. Multicentre trials have shown that the incidence of severe DIARs was thereby considerably reduced (214).

## 5.4. Iron-dextran

The therapeutic value of colloidal iron preparations was first reported in the 1950's by London and Twigg (215). Numerous attempts have been made to improve these iron preparations (216-218). Thus dextran ([η] 0.05) is first heated with alkali, and is then neutralized in the presence of ferric chloride solution. Studies on this product have revealed that each particle consists of a central iron core, approximately 3 nm diameter, surrounded by a dextran sheath of approximately 13 nm diameter (219, 34). The complex is visualized as a particle formed by a protective sheath of dextran attached by terminal metasaccharinic acid units to a β-FeOOH core. A solution of this complex containing 5% iron and 20% dextran (Imferon™) is suitable for intramuscular and intravenous injection for treating iron deficiency anemia. The product is currently used widely for treating anemia in new-born piglets.

The use of these preparations has been re-examined in humans and a dramatic rise in hemoglobin was reported following intravenous infusion. The solution is best administered together with glucose solutions (220–222).

## 5.5. Dextran sulfate

Dextran sulfate (dextran hydrogen sulfate, sodium salt) has been tested as a potential substitute for heparin in anticoagulant therapy (223). Following studies by Ingelman (224), Walton and Ricketts (225–227) explored the anticoagulant properties of a wide range of dextran sulfates ($\overline{M}_W$ 7 000 to 458 000) and established that the lowest molecular weight products displayed the highest anticoagulant properties. However, at best this only represented 15% of heparin's activity.

The toxicity of these products had been recognized at an early stage. Nevertheless, a low molecule weight fraction with $\overline{M}_W$ 7 000 and S, 16% was considered to be qualitatively similar to heparin (228, 229). Preliminary clinical trials were unfortunately discouraging and revealed severe adverse reactions, notably, stiff and painful joints, skin eruption, loss of hair and gastro-intestinal symptoms (230). In chronic toxicity studies in animals, retardation in weight gain and osteoporosis with spontaneous fractures were observed (231, 232). It should be noted that the doses in the clinical trials corresponded to 1.3 g/day, which is approximately tenfold that used in current heparin therapy.

With the introduction of low-dose heparin therapy for thrombosis prophylaxis in the 70's (233–235), interest in low molecular weight polyanions has been rekindled and reports on interactions with individual enzyme/inhibitor systems in the coagulation cascade have appeared (236, 237) (see also 6.5.1).

Dextran sulfate immobilized on cellulose has been found to remove LDL cholesterol preferentially during plasmaphoresis (238).

## 5.6. Diethylaminoethyl dextran (DEAE-dextran)

McKernan and Ricketts (239) originally reported the preparation and properties of DEAE-dextran, A preparation with $\overline{M}_W$ approx. 500 000 has, at a daily oral dose of 2–3 g, been shown to effect a reduction in serum cholesterol and triglycerides by 8 and 14% respectively (240). This dose should be compared with the considerably larger doses (4 x 4 g daily) of the insoluble cationic resins (e.g. Cholestyramine™) which must be taken to achieve comparable effects. Further reports on the pharmacology of DEAE-dextran have appeared (241, 242).

## 5.7. Perfusion solutions

Organ transplantation has in recent years made rapid strides. The need to preserve the viability of transplant organs is urgent. One of the requirements of a perfusion solution is that it must be iso-osmotic with the intracellular fluid. The addition of 5–10% low MW dextran has proved beneficial in preserving isoosmolality and good results have been obtained with kidney, liver, and cornea perfusions (243–247).

## 5.8. Debrisan

Debrisan™ is a wound-cleansing agent prepared in bead form by the emulsion polymerization of dextran with epichlorohydrin. The product acts by absorbing (approx. 4 ml/g) wound exudate in secreting, infected wounds, ulcers and sores, thereby shortening the healing time. The idea, exemplifying one of many serendipitous innovations in the pharmaceutical industry, was conceived in 1972 when Ulf Rothman at Malmö General Hospital, Sweden was investigating the proteins in exudate from apocrine sweat glands in patients suffering from excessive axillary perspiration (248). Exudate was initially collected with filter paper but later Sephadex G-25 was used. One patient with badly infected sores showed a marked improvement after the application of this material. This observation inspired Rothman and his colleagues (249, 250) to initiate further trials on skin wounds and lesions, leading to the development of a pharmaceutical grade cross-linked dextran (Debrisan) which was launched in 1977.

Numerous reports of trials have appeared during the past decade confirming Debrisan's efficacy in promoting wound healing (251–253).

## 5.9. Hyskon

A 32% solution of Dextran 70 stabilized in 10% glucose has proved valuable as a distending medium in operative hysteroscopy (254, 255). It has also been shown to prevent the formation of post-operative tissue adhesions after tubal or abdominal surgery (256–258).

# 6. General applications of dextran and its derivatives

In this section, examples of known commercial applications of dextran products and examples of applications with proven technical or biological effects have been selected. A factor that has restricted the widespread commercial use of dextran is the price, which is somewhat higher than most starch and cellulose products on the market. Dextrans are thus most likely to find application in high quality or high technology products.

## 6.1. Dextran fractions

The demand for technical dextrans from industry has shown a significant increase in the past decade.

Since household sucrose, fruits or fruit beverages could be contaminated with traces of dextrans, ingestion of dextrans, albeit in small amounts, may not be uncommon. Dextran NRRL B-512(F) is degraded by dextran-splitting bacteria in the human gut and most of the hydrolysis products can be absorbed to produce a rapid increase in blood sugar and liver glycogen (259–262).

However, in the food industry, where innumerable applications of dextrans in foodstuffs were patented in the 50's and 60's, no application appears to have been pursued and the mandatory toxicological studies to gain FDA approval were not performed. Hence in 1977, the GRAS (generally recognized as safe) status of dextrans was deleted (263-265). Dextrans are not permitted in the UK or Europe as foodstuff additives, and dextrans do not seem to have been considered by the Joint FAO/WHO Expert Committee on Foodstuff Additives (JECFA). Dextrans are, however, considered as safe as components of food packaging materials.

Dextran fractions do not appear to be included in the lists of permitted additives (ingredients) for pharmaceutical formulations such as ointments and creams for topical use and tablets and capsules for oral use. However, providing the appropriate documentation is presented, there are no *a priori* reasons why they may not be used. Indeed several products in which a dextran fraction is used as a non-active ingredient are on the market.

Purified dextran fractions with high clarity and low chloride levels find extensive applications in the photographic industry. Addition of low concentrations of dextran to the silver emulsion is found to enhance significantly the quality of the images. The effect is presumably attributable to the effect of dextran on the conformation of the gelatin molecules.

Since Albertsson (266) revealed the enormous potential of 2-phase polymer systems, especially dextran-PEG systems, for the partition of sub-cellular particles and macromolecules, an immense number of applications has evolved. These systems offer a means of fractionation beyond the range of conventional techniques. Some recent applications are: the separation of peripheral blood cells (267), distinguishing erythrocytes from multiple sclerosis patients (268), the separation of enzymes, for example pullulanase, from *Klebsiella pneumoniae cells* (269), and the partitioning of murine lymphoblasts (270).

Dextran has been recommended as a cryoprotective agent for human, animal and plant cells (271–273). Thus a mixture of 5% methyl sulphoxide and 9% Dextran 70 was found to afford optimal cryoprotection of human bone marrow committed stem cells (273).

The effect of dextrans as adjuvants for prolonging local anesthetic block has been a matter of some debate. Early results had proved somewhat contradictory (274–276). Recent reexamination by Hassan and colleagues (277–278) has revealed that the prolongation of the effect of anesthetic is dependent on the anesthetic used, the MW of the dextran, and the type of dextran derivative used. A prolongation of up to 350% has been obtained.

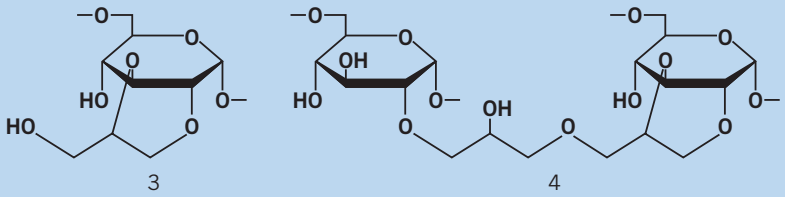
## 6.2. Sephadex

The introduction of Sephadex in 1959 heralded a new era in separation science. The concept of gel permeation chromatography (GPC) developed slowly and the key events leading to the discovery of Sephadex may be traced to Ingelman´s early studies on the cross-linking of dextran, the subsequent patent by Flodin and Ingelman (279) on the preparation of cross-linked gels and the exploitation of their gel filtration properties by Flodin and Porath (280).

Perhaps the most critical event was the synthesis of these gels in bead form with considerably improved flow rates (281), an innovation that led to the immediate acceptance of these gels in separation technology. Several types of Sephadex are currently available, each with a characteristic fractionation range. The most porous gel, Sephadex G-200, will fractionate proteins in the $M_r$ range 4 000–800 000, whereas the upper limit for Sephadex G-25 is 5 000. Although Sephadex gels are widely used for fractionation and purification of biopolymers, desalting constitutes one of the most important applications. In this context, desalting also refers to buffer exchange operations and the removal of low molecular weight impurities other than salts. Sephadex G-25 is now used for desalting or buffer exchange in many large scale operations (282). Insulin producers use Sephadex G-50 to remove proinsulin and protease impurities in the final stages of purification of porcine or bovine insulins (283, 284).

The structure of Sephadex G-25 has recently been investigated by Holmberg (285) and the following novel structural features have come to light.

1. Only 4% of the glucose units are cross-linked.
2. Eight different structural elements were identified, including several glyceryl ethers of glucose and cyclic structures containing 1, 4-dioxane rings. Two of these fragments are represented below 3 and 4.
3. 80% of the beads consists of dextran.




3 4

A number of high capacity anion- and cation-exchangers based on Sephadex have been prepared and are available commercially. The sulphopropyl and quaternized DEAE (QAE) derivatives may be used for separating substances that are only charged at extreme pH's while the DEAE- and carboxymethylderivative can be used for the intermediate ranges.

These gels are now employed commercially for the fractionation of plasma proteins, in particular, human serum albumin, blood clotting factors, immunoglobulin G and haptoglobulin (282) and these chromatograpic techniques are gradually replacing older batch fractionations.

For the separation according to molecular size in organic media, a hydroxylpropyl derivative of Sephadex LH-20 was developed. This facilitates the separation of lipids, hormones, fatty acids etc.

## 6.3. Dextran-hemoglobin preparations

Stroma-free hemoglobin would ostensibly appear to provide the ideal blood substitute. Studies have shown, however, that unmodified hemoglobin is excreted too rapidly by the kidneys (286–287). The preparation and properties of dextran-hemoglobin complexes prepared by consecutive reactions with cyanogen bromide, ethylene diamine, bromoacetylbromide, and finally hemoglobin have been studied (288, 289). A complex containing approx. 1:1 molar proportions of hemoglobin to Dextran 20 was cleared slowly from the circulation (approx. 20% after 4 hours) (290). It was found, however, that the oxygen is bound much more tightly than in hemoglobin and the oxygen delivery potential of these complexes needs to be further explored. Although the complexes were found to be non-immunogenic in homologous species (with regard to the hemoglobin) (291), the risks inherent in activating the immune system to the dextran moiety have yet to be studied. This approach may, nevertheless, enable outdated red cells to be used with advantage (292).

## 6.4. Conjugates of dextran with biologically-active substances

An account of the research in this area, which embraces the coupling of drugs, enzymes, hormones, and antibodies, would merit a chapter to itself. Molteni (293, 294) and Rogovin (295) have surveyed some of the fascinating facets of this topic.

The aims of conjugation to dextran are several:

1. to prolong the lifetime of the active component
2. to increase its stability
3. to facilitate targeting of the drug
4. to depress the antigenicity of the protein moiety

The search for mild coupling procedures has provided ground for much ingenuity. The classical procedure, reported by Porath and coworkers in 1967, on the activation of polysaccharides with cyanogen bromide is still widely used (296-298). The mechanism is depicted in Figure 10.

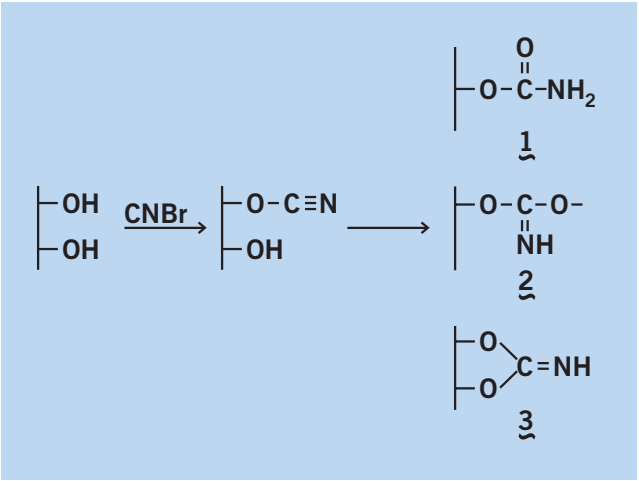



**Fig 10.** Activation of dextran yielding the intermediate structures 1, carbamate 2, linear imidicarbonate and 3, cyclic imidocarbonate.
The latter is the most reactive.

Many alternative coupling procedures have since been devised, for example activation via 1,1'-carbonyldiimidazole (299), reductive amination (300), isocyanides (301) and cyclic carbonates (302, 303).

Marshall (304) has reviewed the preservation of enzymes by coupling to dextran. Studies on conjugates between dextran and α-amylase or trypsin have established that the conjugates acquire increased resistance to heat inactivation, to proteinases and to the action of inhibitors. The lifetime of an asparaginase-dextran 70 conjugate was prolonged 20-fold, *in vivo*, whereby a patient with lymphoblastic leukemia was able to deplete plasma asparaginase for approx. 100 days compared to 7 days for asparaginase itself (305). Conjugates between dextran and a number of serum peptidases have been prepared, e.g. carboxypeptidase G2 (306, 307), kallikrein (308), and fibrinolysin (309).

The conjugation of cytostatics to dextran has been explored and, in many cases, these conjugates displayed a high cellular uptake, good activity and less toxicity. These effects are, in part, attributable to the preferential uptake of dextran by lysosomes and the higher endocytic activity of tumour cells (310). The coupling of daunomycin (311, 312), methotrexate (313), daunorubicin (314), mitomycin C (315-317) and 7-deazaguanine (318) has been described.

Larsen (319, 320) has used O-benzoyl dextran derivatives as model compounds for studying the kinetics and mechanism of ester hydrolysis in plasma. It was concluded that the hydrolysis is not mediated by enzymes. Factors affecting the release of metronidazole and naproxen from dextran conjugates have been investigated (321-323).

Successful attempts to couple insulin to dextran have been reported (324, 325). The hypoglycemic action of the complex is claimed to be superior and more prolonged than that of insulin itself.

The coupling of ragweed pollen Antigen A to dextran has reduced the allergenicity and antigenicity of the allergen by a factor of 7 (on a molar basis). This effect was, however, insufficient to warrant further clinical studies (326).

Before the clinical potential of these devices can be fully exploited, the safety of these compounds must be documented.

1. How does the substitution affect the metabolism and toxicity of the carrier? It is known that even low degrees of substitution may seriously retard the metabolism and elimination from the body.
2. Is the immunogencity of the complex enhanced (327)?
3. Can sufficient active substance be coupled to the dextran so that therapeutic levels can be attained without using excessively high concentrations of the conjugate?

## 6.5. Dextran derivatives

### 6.5.1. Dextran sulfate

Some reference to this substance in connection with its effects on coagulation has already been made in section 5.5. Its wide spectrum of biological properties has attracted the attention of many scientists. The reports may be grouped according to the following biological effects.

a. Enzyme inhibition, activation and release
b. Adjuvant effect on immune response
c. Cellular interactions and response
d. Effect on virus infectivity

This classification is necessarily rather arbitrary as, in many cases, the observed biological effects are the result of two more of these activities. The studies pertaining to enzymes constitute by far the largest group with approximately half demonstrating inhibitory effects and half, activating effects.

In each particular instance, it must be stressed that the observed effects are dependent not only on the MW and DS of the dextran sulfate but also on concentration, pH and ionic strength. Unless stated otherwise, a dextran sulfate with $\overline{M}_W$ of 500 000 and high DS approx. 2 has been used in most of the studies.

#### a. Effects on enzymes

Inhibitory effects have been reported for, *inter alia*, hyaluronidase (328), LH-sensitive adenylate cyclase (329), $PGE_1$-sensitive adenylate cyclase (330, 331), human α-glucosidase (332), ornithine decarboxylase which plays a key role in cell growth (333), α-amylase and bacterial dextranase (334).

Dextran sulfates are potent non-competitive inhibitors of human leucocyte elastase, an enzyme that may be implicated in cartilage degeneration (335). The activity appeared to increase with increasing MW and DS. Reports on the activation of enzymes by dextran sulfates are equally numerous. Whether we are dealing here with the inhibition of an inhibitor in the system is uncertain.

Dextran sulfate appears to stimulate the following enzymes, AMP-dependent rabbit muscle phosphorylase b (336), tyrosine-3-monoxygenase (337), urokinase (338), calium-dependent ATP-ase (339), and rat brain tryptophan hydroxylase (340).

Numerous studies on the coagulation cascade show that both activation and inhibition may be operative. Thus dextran sulfate has been found to activate the polymerization of fibrin monomer (341), ATIII (342), conversion of prekallikrein to kallikrein (343–345) and fibrinolysis (346, 347). However, kallikrein (344), the conversion of fibrinogen to fibrin (341) and thrombin (348, 349) appear to be inhibited by dextran sulfate. These effects may be, *inter alia*, concentration dependent.

### b. Effects on immune response

Many reports serve to illustrate that dextran sulfate interferes in many ways with the immune response. Evidence that it is a B-cell mitogen stimulating the proliferation of B cells and thereby the immune response has been presented (350–354). However, *in vivo*, dextran sulfate (i.p., 50 mg/kg) has been found to increase the susceptibility of mice to bacterial infection (355–357). These observations have been ascribed to the toxic effects of sulfate on the mononuclear phagocytes (357, 358). Even at low concentrations (20 µg/ml), exposure of macrophages to dextran sulfate leads to an accumulation within the secondary lysosomes and an inhibition of the phagosome-lysosome interaction fusion (354–361) and may thus interfere with the enzymes responsible for killing bacteria (362). Increased permeability of the lymphocytes with excessive leakage of low MW components may also be an important factor (363–365). Dextran sulfate also induces the mobilization of B and T lymphocytes *in vivo* (366–369).

### c. Effects on viruses

The reports on the effects of dextran sulfate on virus infectivity and growth (as with tumour growth) are conflicting. Variable effects are noted with foot and mouth viruses (370). Herpes simplex viruses were inhibited (371). Although dextran sulfate inhibits the release of virions from cells (372), a combination of lipopolysaccharide and dextran sulfate induces their release from mouse spleen cells (373). *In vivo*, the resistance of experimental animals to viral infection was potentiated by dextran sulfate (374, 375).

In 1987, the antiviral activity of dextran sulfate against human immunodeficiency virus HIV-1 was established (376, 377). The optimal activity, *in vitro*, is obtained with a molecular weight ($\overline{M}_W$) of approx. 10 000 and DS 2 (378, 379). Dextran sulfate with $\overline{M}_W$ of approx. 7000 is, however, poorly adsorbed from the gut (380) and it is uncertain whether therapeutic concentrations can be obtained by this route.

### d. Toxicity

The toxicity of a low MW dextran sulfate, administered intravenously, has been referred to under Section 5.5. More recently attention has been focussed on the toxicity of orally administered substance. Supplements of 0.25% and 0.5% for 82 weeks in rats did not increase the incidence of infection or tumours (381). Higher doses (1–10%) resulted in mortality and tumours (382, 383). Apart from dosage, the toxicity is also dependent on MW (384).

Adsorption studies in the gut using a wide range of dextran sulfates (3 000–200 000) suggest only a small proportion of the dose is absorbed (385–387). Intravenous administration has also been investigated (388). The inhibition of metastasis by dextran sulfate ($\overline{M}_W$ 6–7 x $10^3$, DS approx. 2) has been tested clinically but the 5 year survival rate in lung cancer was not improved (389, 390). Tests for mutagenicity and cytogenicity of low MW dextran sulfate proved negative (391).

### e. Gene manipulation

Finally, some mention should be made of the successful application of dextran sulfate in gene manipulation, where it has become a reagent of particular value for accelerating the hybridization of DNA fragments (392–394); for accelerating the transfer of DNA fragments from agarose gels (394); and for detecting recombinant mammalian viruses in plaques (395).

## 6.5.2. DEAE-dextran

The reaction of diethylaminoethyl chloride with dextran in alkali affords a derivative in which three kinds of charged amine groups can be distinguished (396, 397): the single DEAE substituent with $pK_a$ 9.2; the tertiary group with $pK_a$ 5.5; and the quaternary group on the tandem group which is dissociated at all pH values.

Like the polyanions, the polycations also exhibit a wide range of interesting biological properties. In particular, DEAE-dextrans appear to enhance a number of cellular processes. Many reports (398-401) testify to the enhancement of cellular uptake of viral RNA and also intact virus particles. Considerable interest has been focussed on the enhanced production of interferon by polyribonucleotide complexes in the presence of DEAE-dextran (402–404). These processes appear to operate without detrimental effects on the viability of the cells (398). To what extent these effects are due to the interaction between the cell surface (net negative charge) and the polycation is uncertain. For most of these studies, a DEAE-dextran with $\overline{M}_W$ approx. 500 000 and DS 0.5 has been used at concentrations from 10–500 µg/ml.

Reports on its effects on tumour growth are contradictory. Larsen and Thorling (405) found that incubation of 4 different types of tumour cells with DEAE-dextran prevented the proliferation of cells in experimental animals. However, Mincheva (406) found no significant effects on metastasis of pulmonary carcinoma in rats. Rice and coworkers (407) reported that repeated injections of DEAE-dextran (500 µg) in mice induced sarcomas.

## 6.5.3. Fluorescein-labelled dextrans

Fluorescein isothiocyanate reacts with dextran to give a fluorescent derivative (408) which has proved useful as a macromolecular tracer in studies of microcirculation and, in particular, vascular permeability in health and disease.

Techniques developed in the 70's (409–412) have been exploited to study permeabilities of brain capillaries (413), pancreatic duct (414), cornea (415), retinal vasculature (416), and skeletal muscle (417).

## 6.6. Microcarriers for cell culture

In 1967, van Wezel (418) reported the use of DEAE-Sephadex A-50 as a microcarrier for culturing anchorage-dependent animal cells. However, these microspheres were not ideal for the growth of cells especially at higher microcarrier concentrations (>2 mg/ml). Subsequent studies (419–422) established that the optimal degree of substitution for cell growth was approx. 1.5 meq/g which was considerably lower than that of the ion-exchanger then available. Several microcarriers based on cross-linked dextran are now available commercially: (1) A microcarrier with DEAE-groups distributed throughout the matrix (Cytodex™ 1); and (2) a microcarrier to which a layer of collagen is attached (Cytodex™ 3). Microcarrier technology is now employed commercially for the production of, amongst others, viruses, vaccines, and cell products such as interferon.

## 6.7. $^{99m}$Tc-Dextran

Technetium appears to complex strongly with dextran and as such has been tested as an agent for lymphoscintigraphy with promising results in the leg, pelvic and para-aortic lymph-nodes (423-425). The radio-labelled pertechnate is first reduced with stannous ion and then added to dextran to yield the technetium complex.

# 7. Dextran analysis

The choice of method will be dictated by the sensitivity required, the nature of the sample and, possibly, by the molecular weight of the dextran present.

In the sugar industry, the alcohol haze method is commonly used (426, 427) but may be replaced by a more specific copper method (428).

Many methods have been devised to measure clinical dextrans in serum and urine. The methods generally involve the removal of proteins by picric acid (429) or trichloroacetic acid (430) followed by the precipitation of dextran with alkaline copper reagent (430), o-toluidine-acetic acid (431), or ethanol (429).

Dextran may then be determined by the anthrone reagent (429, 432), turbidimetrically (433), polarimetrically (434) or as reducing sugar after hydrolysis (431). Numerous refinements to these methods have appeared in the literature (435-437). A procedure employing glucose oxidase has been described (438).

A specific and sensitive immunochemical determination has been developed which detects dextran concentrations down to 2.5 µg/ml (439).

# 8. Acknowledgements

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# Ordering information

## Native Dextran

| Product | Pack size | Code No. |
|---|---|---|
| **Native Dextran Powder** | 20 kg | 30-0464-10 |
| **Native Dextran Solution (Syrup)** | 1000 l IBC* | 30-0464-20 |

*IBC = International Bulk Container

## Technical Dextran

| Product | Pack size | Code No. |
|---|---|---|
| **T10P** | 40 kg | 10-4276-01 |
| **T20P** | 40 kg | 17-0260-07 |
| **T40P** | 40 kg | 17-5102-07 |
| **T45P** | 40 kg | 17-5188-01 |
| **T70P** | 40 kg | 17-0281-01 |

## Dextran Fractions

| Product | Pack size | Code No. |
|---|---|---|
| **Dextran 1** ($M_W$ 1000) | 5 kg | 10-1663-05 |
| | 30 kg | 10-1663-01 |
| For larger volumes, please contact your local representative | | |
| **Dextran 3,5** ($M_W$ 3500) | 40 kg | 10-1657-01 |
| Dextran 5 ($M_W$ 5000) | 100 g | 10-1654-04 |
| | 500 g | 10-1654-05 |
| | 5 kg | 10-1654-06 |
| For larger volumes, please contact your local representative | | |
| **Dextran 10** ($M_W$ 10 000) | 100 g | 17-0250-01 |
| | 500 g | 17-0250-02 |
| | 5 kg | 17-0250-03 |
| For larger volumes, please contact your local representative | | |
| **Dextran 20** ($M_W$ 20 000) | 100 g | 17-5239-01 |
| | 500 g | 17-5239-02 |
| | 5 kg | 17-5239-03 |
| For larger volumes, please contact your local representative | | |
| **Dextran 40** ($M_W$ 40 000) | 100 g | 17-0270-01 |
| | 500 g | 17-0270-02 |
| | 5 kg | 17-0270-03 |
| For larger volumes, please contact your local representative | | |
| **Dextran 70** ($M_W$ 70 000) | 100 g | 17-0280-01 |
| | 500 g | 17-0280-02 |
| | 5 kg | 17-0280-03 |
| For larger volumes, please contact your local representative | | |
| **Dextran 110** ($M_W$ 110 000) | 500 g | 17-0290-02 |
| | 5 kg | 17-0290-03 |
| For larger volumes, please contact your local representative | | |
| **Dextran 500** ($M_W$ 500 000) | 100 g | 17-0320-01 |
| | 500 g | 17-0320-02 |
| | 5 kg | 17-0320-03 |
| For larger volumes, please contact your local representative | | |
| **Dextran 2000** ($M_W$ 2 000 000) | 100 g | 17-0330-01 |
| | 500 g | 17-0330-02 |
| | 5 kg | 17-0330-03 |
| For larger volumes, please contact you local representative | | |
| **Dextran CB** | 40 kg | 10-1659-01 |

## Clinical Dextrans

| Product | Pack size | Code No. |
|---|---|---|
| **Dextran 1 Substance** | 5 kg | 10-1663-05 |
| | 30 kg | 10-1663-01 |
| NDC* no. 63297-841-30 | 30 kg | 10-1663-02 USA |
| For larger volumes, quotation on request | | |
| **Dextran 10 Substance** | 100 g | 17-0251-01 |
| | 500 g | 17-0251-02 |
| | 5 kg | 17-0251-03 |
| | 40 kg | 17-0251-07 |
| For larger volumes, quotation on request | | |
| **Dextran 40 Ph Eur** | 5 kg | 10-4309-06 |
| | 20 kg | 10-4309-04 |
| | 40 kg | 10-4309-01 |
| For larger volumes, quotation on request | | |
| **Dextran 40 USP** | | |
| NDC* no. 63297-821-05 | 5 kg | 10-4309-05 |
| NDC* no. 63297-821-20 | 20 kg | 10-4309-03 |
| NDC* no. 63297-821-40 | 40 kg | 10-4309-02 |
| For larger volumes, quotation on request | | |
| **Dextran 60 Ph Eur** | 40 kg | 10-4280-01 |
| **Dextran 70 Ph Eur** | 5 kg | 10-4279-06 |
| | 20 kg | 10-4279-04 |
| | 40 kg | 10-4279-01 |
| For larger volumes, quotation on request | | |
| **Dextran 70 USP** | | |
| NDC* no. 63297-811-05 | 5 kg | 10-4279-05 |
| NDC* no. 63297-811-20 | 20 kg | 10-4279-03 |
| NDC* no. 63297-811-40 | 40 kg | 10-4279-02 |
| **Dextran 40 JP Substance** | 40 kg | 10-4310-01 |
| **Dextran 70 JP Substance** | 40 kg | 10-4278-01 |

*USA NDC: National Drug Code

## Dextran Sulphate sodium salt

| | Pack size | Code No. |
|---|---|---|
| | 100 g | 17-0340-01 |
| | 500 g | 17-0340-02 |
| | 1 kg | 17-0340-08 |
| | 5 kg | 17-0340-03 |
| | 40 kg | 17-0340-20 |

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## Dextran Sulphate 10 sodium salt

| | Pack size | Code No. |
|---|---|---|
| | 1 kg | 17-0345-08 |
| | 5 kg | 17-0345-03 |

## DEAE-Dextran

| Pack size | Code No. |
|---|---|
| 100 g | 17-0350-01 |
| 500 g | 17-0350-02 |
| 1 kg | 17-0350-09 |
| 5 kg | 17-0350-03 |

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