has shown that the immediate mortality is low, and that most patients derive great benefit from operation.

It is gratifying to learn from family doctors and from relations that for many of these women the last few months or years of life were made much more comfortable by plastic repair operations.

We are grateful to our consultant colleagues, Mr. Frank Stabler, Mr. Derek Tacchi, and Mr. Denys Fairweather, for permission to publish details about cases admitted under their care.

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Molecular Composition of Transfusion Dextran

A Report to the Medical Research Council's Blood Transfusion Research Committee*

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Brit. med. J., 1966, 2, 1423–1426

The persistence of dextran in the circulation after its intravenous injection depends upon the molecular-weight distribution; smaller molecules are quickly excreted, while larger ones stay and maintain the plasma volume. As molecular weight increases the interaction of dextran molecules with proteins and cells increases. Allergic reactions and increases in bleeding-time have been ascribed to the larger molecules. Moreover, dextran molecules large enough to remain in the circulation for what may be considered to be a useful time tend to cause red cells to adhere together, forming rouleaux.

The dextran used in this country, dextran *B.P.*, has a higher average molecular weight (150,000) than is advocated elsewhere. This confers the advantage of ensuring a satisfactorily long duration of plasma-volume expansion, but it does carry with it the disadvantages referred to in the previous paragraph. Whether or not increased aggregation of red cells is a serious disadvantage remains undecided after 10 years or more of using this dextran.

In the past few years, however, observations of blood-flow in animals have underlined the possible ill effects (Gelin and Zederfeldt, 1961) of high concentrations of dextran of high molecular weight upon the free flow of blood in capillaries through excessive rouleaux formation (Thorsén and Hint, 1950). Microcirculatory and histological investigations have been made on the aggregation of red cells by dextrans (Stalker, 1961, 1964). "Sludging" of the blood and blocking of capillaries have been observed and recorded on cine-film in rabbits, both normal and shocked, which had been given dextran *B.P.*, but these effects occurred to a much less degree after infusion of the dextrans of lower average molecular weight used elsewhere. The degree of aggregation produced by dextran *B.P.* caused significant histological damage of hypoxic type, particularly in the liver and myocardium, while the dextrans of lower molecular weight caused little or no damage. It is not known whether similar changes occur in man, or, if they do occur, whether they are of any clinical significance.

"Sludging"

In the interest of obtaining a satisfactory persistence in the circulation it is possible that too much "sludging" has been accepted. What could be done to minimize sludging while still retaining a satisfactory persistence in the circulation? A quantitative answer can be obtained from previous experimental work.

No way is known of assessing quantitatively the sludging which occurs *in vivo*. *In vitro* rouleaux formation causes an increase in the rate of sedimentation of red cells from a suspension. This rate measured under standardized conditions is the simplest *quantitative* measurement of the effect of dextran on red cells. It is well known that the larger dextran molecules have the biggest effect on the red-cell sedimentation rate, and in Fig. 1 some earlier data (Hardwicke *et al.*, 1950) are replotted to illustrate that dextrans with intrinsic viscosity below about 0.25 have no effect on sedimentation rate, but above this there

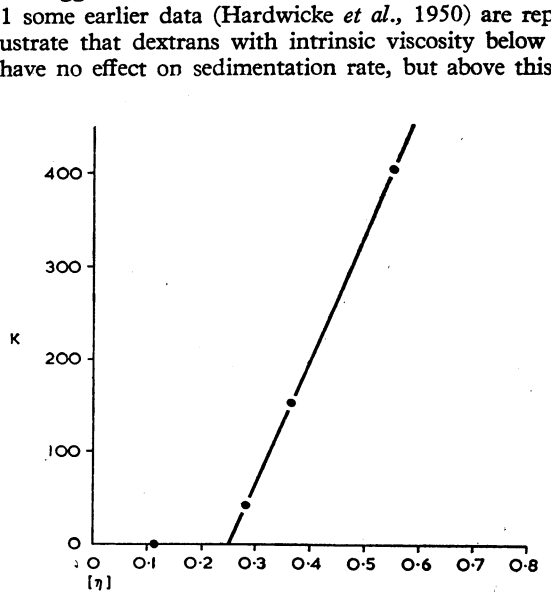

FIG. 1.—Relation between red-cell sedimentation rate (K) and intrinsic viscosity (η) of dextran. Hardwicke and Squire (1952). Calculated log. K for dextran fractions with the intrinsic viscosities shown.

* Members of the Committee were: Professor P. L. Mollison (chairman), Dr. J. P. Bull, Dr. R. J. Drummond, Sir Alan Drury, Dr. R. A. Kekwick, Dr. J. C. Kelsey, Dr. J. F. Loutit, Professor R. G. Macfarlane, Professor M. Maizels, Dr. W. d'A. Maycock, Dr. A. E. Mourant, Professor W. D. M. Paton, Professor T. A. J. Prankerd, Dr. J. Wallace, Dr. K. L. G. Goldsmith (secretary).
The work was done with the collaboration of Mr. A. J. Evans and Dr. J. Kohn, Queen Mary's Hospital, Roehampton; Dr. J. P. Bull, Mr. J. Cason, and Mr. D. Jackson, M.R.C. Burns Research Unit; Dr. W. d'A. Maycock, Lister Institute, Elstree; and Dr. A. L. Stalker, Department of Pathology, University of Aberdeen.

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is a steeply increasing effect with increasing intrinsic viscosity. "K" was defined as the corrected maximum sedimentation velocity at 1% dextran concentration measured under standardized conditions. It was also shown that the effects of mixtures of dextran fractions on red-cell sedimentation were in proportion to the amount of each fraction present (Hardwicke and Squire, 1952).

Consider now a sample of dextran *B.P.* which has been fractionated into 14 fractions (Rowe, 1956). Table I shows the percentage by weight of each fraction and the inherent viscosity of each fraction (for the purpose of this discussion inherent viscosity may be assumed to be identical with intrinsic viscosity). The fourth column of the Table shows the contribution which each fraction makes towards the inherent viscosity of the whole polymer. Experiments have shown that such contributions are additive and may be used to calculate the inherent viscosity of a mixture of fractions. The next column shows the K value from Fig. 1 corresponding with the inherent viscosity of each fraction.

TABLE I.—*Contribution of Each Fraction of Dextran B.P. to the Red-cell Sedimenting Effect of the Whole*

Fraction No.	% by Weight	Inherent Viscosity	Viscosity Contribution	K
1	10·97	0·4178	0·04583	240
2	6·13	0·3941	0·02415	195
3	5·46	0·3730	0·02036	165
4	5·88	0·3610	0·02122	155
1 to 4 total	28·44			
5	3·78	0·3426	0·01295	125
6	6·30	0·3429	0·02160	125
5 and 6	10·08	(0·34)		
7	4·34	0·3276	0·01421	110
8	6·10	0·3159	0·01926	90
9	11·17	0·3079	0·03439	78
10	7·03	0·2922	0·02054	60
11	8·72	0·2750	0·02398	30
12	6·30	0·2510	0·01581	5
13	13·61	0·2231	0·03036	0
14	4·14	0·1869	0·00773	0
5 to 14 total	71·49	(0·28)		
1 to 14 total	99·93		0·31239	

Inherent viscosity closely approximates to intrinsic viscosity but is calculated from a measurement of relative viscosity at one concentration. The inherent viscosity of the whole is 0·31, of fractions 5 to 14 it is 0·28, and of fractions 5 + 6—i.e., the top 14% of fractions 5 to 14—it is 0·34.

The effect of fractioning the dextran to any desired extent may now be seen. For instance, it is possible to remove in one step the first four fractions, which total some 28% by weight, thus removing those molecules which make the greatest contribution, probably more than 50%, to red-cell aggregation. The corresponding changes in intrinsic viscosity have been calculated and are shown in Table I. The persistence in the human circulation of dextrans of various molecular weights was measured by Howard *et al.* (1956), who found little difference between fractions of average molecular weight 194,000, 255,000, and 412,000. The top 30% of dextran *B.P.* includes material with these molecular weights. There is therefore reason to believe that sludging could be minimized by removing approximately 30% from the upper end of the molecular-weight range of dextran *B.P.* without greatly affecting its persistence in the circulation.

TABLE II.—*Characteristics of Transfusion Dextrans*

	Dextran *B.P.* Limits	Proposed Values*	Measured Values	
			Batch G	Batch B
"Top" fraction size (%) and intrinsic viscosity	(<10%) <0·50	(14%) 0·34	(9·4%) 0·328	(6%) 0·38
Whole intrinsic viscosity	0·29 to 0·35	0·28	0·275	0·29
Renal excretion, percentage of dose excreted in 48 hours	<25	<25	28·1	26·9

* For a single batch of modified dextran derived from Table I.

Table II shows relevant features of the specification for dextran *B.P.* and some proposed values for a modified dextran, taking into account the foregoing considerations.

Experimental Work

Under the aegis of the M.R.C. Blood Transfusion Research Committee the manufacturers of dextran *B.P.* were invited to prepare for clinical use two batches of modified dextran; measurements made on these batches, G and B, are also shown in Table II. As might be expected, it proved difficult to reduce the average molecular weight without at the same time exceeding the 25% limit for renal excretion laid down by the *B.P.*; nevertheless, the technical difficulties of fractionation on a large scale were satisfactorily overcome. Except in respect of molecular size the solutions of modified dextran conformed to the *B.P.* specification.

Simple experiments *in vitro* showed that the effect of the modified dextrans upon red-cell sedimentation rate was substantially less than that of the dextran *B.P.* in current use (see Fig. 2).

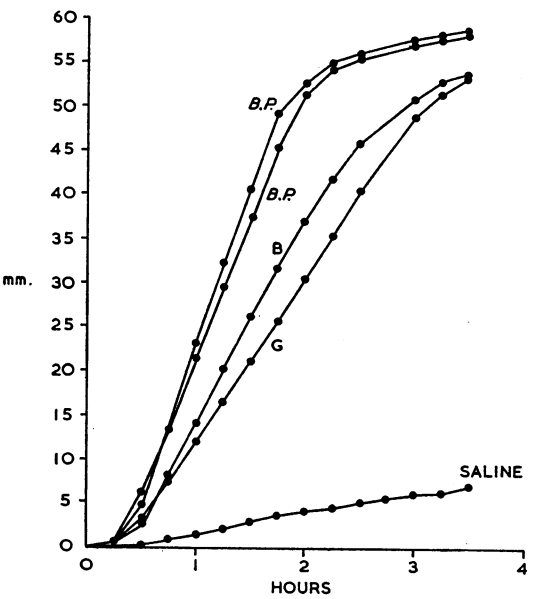

FIG. 2.—Effect upon red-cell sedimentation rate of two batches of dextran *B.P.* and two batches of modified dextran. The mixture contained 1 ml. human blood plus 0.5 ml. 1.6% dextran. Sedimentation was measured in a Wintrobe tube at room temperature.

The microcirculation visible in a rabbit ear chamber was observed in rabbits from which 60 ml. of blood had been withdrawn and replaced with 60 ml. of the modified dextran solutions. Observations were made after five minutes, one hour, six hours, and 18 hours, and thereafter daily for five days. Slight red-cell aggregation appeared quickly and worsened until after six hours small aggregates of 2 to 6 red cells were seen in capillary and venular channels. There was never any more severe aggregation than this, and no interference with paracapillary blood-flow could be seen. There was no "trapping" of red cells in capillaries and no impaction of red-cell masses at junctions.

If these results are compared with those obtained by using narrow fractions of dextran the degree of aggregation was slightly greater than that caused by dextran of Mw 75,100, and notably less than that caused by dextran Mw 153,000.

A search for histological damage to lung, kidney, adrenal, liver, and myocardium was made in 10 rabbits subjected to haemoconcentration by subcutaneous infiltration under anaesthesia of 40 ml. of 50% dextrose. All were in moderate shock

after two hours, and were given 20 ml. modified dextran solution per kg. body weight. They were killed after 48 hours. Slight histological changes (trivial round-cell infiltration or focal necrosis) were observed in the myocardium and liver in about half the animals. These changes were notably less in incidence and degree than those observed after transfusion of dextran *B.P.*

The persistence in the circulation and the magnitude and duration of plasma-volume expansion were measured in an experiment of cross-over design in rabbits. The performance of the two modified dextrans was compared with that of two *B.P.* dextrans. Four rabbits were used, each receiving a modified dextran, followed after a suitable interval by a *B.P.* dextran or vice versa, Before each measurement ^{125}I-labelled rabbit albumin was allowed to equilibrate in the animal, and changes in plasma volume were deduced from changes in plasma radioactivity. Results were averaged for the modified and for the *B.P.* dextrans, thereby minimizing rabbit-to-rabbit and other experimental differences. The modified dextran appeared to leave the circulation a little more quickly than dextran *B.P.* (see Fig. 3). The magnitude and duration of plasma-volume expansion are shown in Fig. 4 ; the original plasma volume of the rabbits was about 110 ml., and this is shown as 100% on the graph. The initial expansion was slightly more than the volume injected, and some degree of expansion lasted for two or three days for both the modified and the *B.P.* dextrans. Differences between the dextrans are probably not significant owing to the limited accuracy of this kind of experiment.

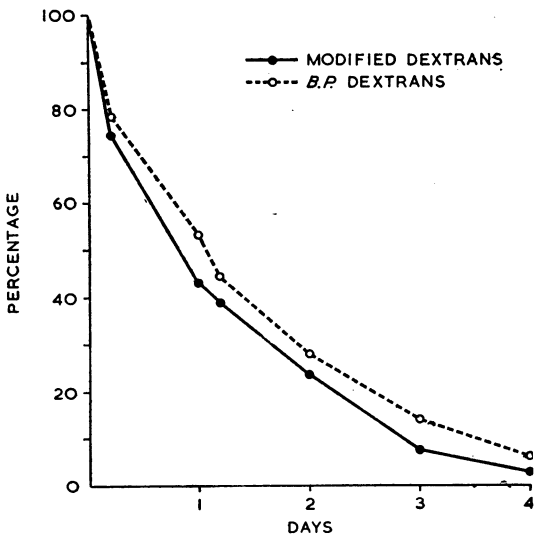

FIG. 3.—Comparison of the persistence in the circulation of rabbits of modified dextrans with *B.P.* dextrans. The concentration of dextran in plasma is plotted as a percentage of the value 10 minutes after injection. Injections were each 30 ml. 6% solution.

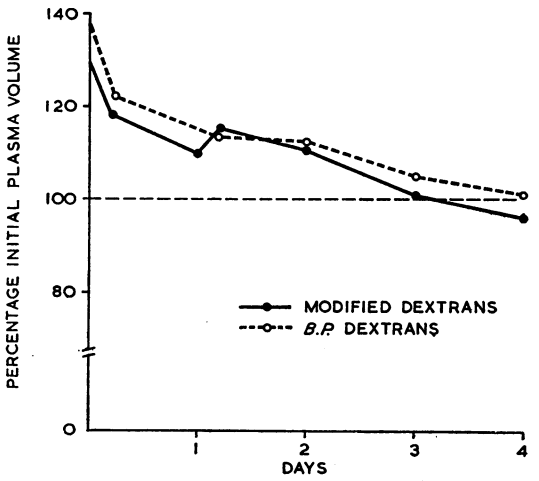

FIG. 4.—Plasma-volume expansion after the infusion of modified dextrans and *B.P.* dextrans as described in Fig. 3.

The viscosity measurements confirmed that the modified dextran contained less material of high molecular weight. The effects of this modification were measurable *in vitro* as a reduction of red-cell sedimentation rate and observable *in vivo* in the capillary blood circulation of the rabbit's ear. Persistence of dextran in circulation, the proportion subject to renal excretion, and the magnitude and duration of plasma-volume expansion were substantially unaltered. There was therefore reason to believe that the modified dextran represented an improvement on the currently used dextran *B.P.*

Clinical Trial

Before recommending an alteration in the molecular-weight distribution it seemed desirable that dextran solutions with the proposed new composition should be used by clinicians with previous experience of dextran under conditions of careful observation. The purposes of this trial were: (1) to collect opinions on the effectiveness, judged clinically, of the solutions as plasma-volume expanders ; (2) to measure the amount of dextran in blood and urine at various times after transfusion ; (3) to observe whether transfusion of these solutions causes any difficulty in blood grouping and cross-matching of subsequently collected blood samples.

An important use of plasma and therefore of plasma substitutes is in the treatment of burned patients. Some of the new dextran was used in the treatment of burned patients at Queen Mary's Hospital, Roehampton, where dextran has been in regular use for many years. The new dextran was also used in the Burns Unit of Birmingham Accident Hospital, where freeze-dried plasma is generally used ; here the volume of dextran transfused was limited to the supposed normal plasma volume of the patients, and any further colloid required was provided as plasma. Table III summarizes the age of the patients, the area burned, and the colloid transfusions given. Cases 1 to 3 were at Roehampton, the others were in Birmingham. In addition to those listed in Table III, 19 other burned patients (in Birmingham) received a total of 26 litres of the new dextran solutions.

TABLE III.—*Details of Patients and Transfusions Given to Them*

Case No.	Age (years)	Area Burned	Dextran (ml.)	Colloid-infused Plasma (ml.)	Blood (ml.)
1	24	37	4,020 G	—	500
2	5	20	950 B	—	250
3	52	23	2,160 G	—	1,080
4	10	20 (14)	1,100 G	1,335	—
5	3	19 (11)	895 G	1,820	—
6	1½	16 (4½)	460 G	485	—
7	3	52	725 B	1,605	250
8	1½	15 (1)	460 B	450	—
9	2½	16 (15)	475 B	145	—

Figures in parentheses denote whole-thickness skin loss.

The effectiveness of a plasma-volume expander is not easy to judge clinically. Surgeons were asked if the expansion of plasma volume had appeared adequate, and whether the expansion had lasted long enough. Unequivocal replies in the affirmative to both of these questions were given in respect of Cases 1 to 3. In Cases 4 to 9, in which plasma was given immediately after dextran, any deficiency of the dextran would be more difficult to detect ; the clinical response as regards the haematocrit reading, urinary output, and general condition of the patient was considered satisfactory by the surgeons.

Fig. 5 shows the serum dextran concentration expressed as a percentage of the concentration measured 10 minutes after the end of transfusion and plotted on a logarithmic scale against time. As might be expected, the group of patients (Cases 4,

7, and 8) who received a substantial volume of plasma immediately after the dextran showed a rapid fall of dextran concentration, which was accompanied by a correspondingly rapid rise of plasma protein. Cases 1, 3, and 9, who received *small* volumes of plasma, either as whole blood or as dried plasma, showed a fall in serum dextran concentration which accorded well with the decrement of one-third per day previously observed (Bull *et al.*, 1949).

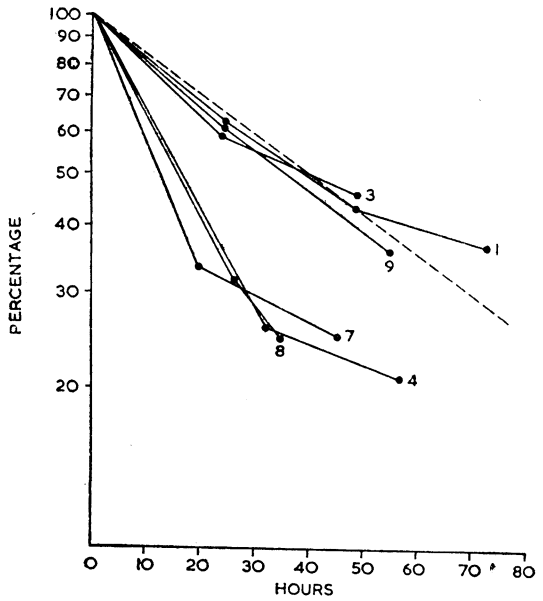

FIG. 5.—Serum dextran concentrations in patients expressed as a percentage of the concentration measured 10 minutes after the end of transfusion and plotted on a logarithmic scale against time. Dextran was estimated by the methods of Davies *et al.* (1963). The broken line denotes a decrement of one-third per day. The increased initial slope in Cases 4, 7, and 8 was due to plasma-volume expansion following plasma transfusion.

Table IV shows the percentage of the dose excreted in the urine in the first three days after transfusion. (Dextran was determined polarimetrically with the appropriate correction for

TABLE IV.—*Renal Excretion of Dextran*

Dextran:	G			B	
Case No.	1	3	5	2	7
Percentage excreted..	25·0	23·9	19·8	20·0	21·7

any glucose present.) These percentages, which might be lower than the percentages actually excreted because of incomplete urine collection or through loss of dextran into exudate from the burned area, are comparable with the renal excretion of 25 to 26% measured in rabbits.

In neither of the patients (Cases 1 and 3) who received the largest volumes of dextran solution, 4 and 2 litres respectively, was there any clinical indication of a raised bleeding-time.

In carrying out blood-grouping tests or cross-matching tests on blood containing dextran there are no problems provided that red cells which have been exposed to dextran-containing serum are first washed. For example, if the indirect antiglobulin test is used for cross-matching donor's red cells against the serum of a patient who has received dextran *B.P.* no problems are encountered (Bull *et al.*, 1949). When agglutination tests are used, as when saline suspensions of red cells are incubated with serum from a patient, excessive rouleaux formation may cause difficulties; suspension of red cells in concentrated albumin rather than saline greatly diminishes the rouleaux formation (Marston, 1954). In the present series the two sera with the highest dextran concentrations (2.33 and 2.86 g./100 ml). could not be cross-matched satisfactorily without albumin. All other samples could be cross-matched satisfactorily in saline, either at room temperature or at 37° C.

Summary

So far as can be ascertained by clinical observations of this kind the proposed new composition for dextran provides satisfactory plasma-volume expansion. The dextran stayed in the circulation about as long as the dextran previously used, and renal excretion was satisfactorily low. The diminished tendency of the modified dextran to form rouleaux led to fewer difficuties in cross-matching.

Miss S. Farrow and Dr. J. Davies, of the M.R.C. Industrial Injuries and Burns Research Unit, carried out the plasma-volume measurements in rabbits. The help in the laboratory of Mr. M. Hall, Mr. H. A. Lilly, and Miss B. Bennett is gratefully acknowledged. Thanks are due to Fisons Pharmaceuticals Ltd. (Benger Laboratories) and Glaxo Laboratories Ltd. for their ready response to suggestions for altering the molecular-weight distribution of dextran and for supplying the modified dextrans for clinical use.

Throughout this report dextran *B.P.* refers to the monograph entitled "Dextran Injection" in the *British Pharmacopoeia* 1963; this material has recently been designated Dextran 150. Modified dextran is described in the *British Pharmacopoeia Addendum* 1966 as Dextran 110.

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