Urinary excretion kinetics of hydroxyethyl starch 350/0.60 in normovolaemic man

JOHN MILTON MISHLER,* COLIN R RICKETTS,† AND EDWARD J PARKHOUSE†

From the *Medizinische Universitätsklinik Köln, Köln, Bundesrepublik Deutschland and the †MRC Industrial Injuries and Burns Unit, Birmingham Accident Hospital, Birmingham, UK

SUMMARY Rates of urinary excretion concomitant with the molecular size distribution of filtered polymer fragments were determined in five normovolaemic men dosed with 30 g/m² BSA of a species of HES (HES 350/0.60) possessing a M_w^- of 350 000 combined with an MS of 0.60. Approximately 13% of the total injected dose of HES 350/0.60 was excreted in urine 1 hour after dosing, and 45% by 72 hours. Gel filtration on Sepharose CL-4B revealed that aliquots of urine collected 1 hour after injection contained polymer fragments of HES 350/0.60 with values of K_{av} ranging between 0.88 and 0.84, and possessed a Stokes radius ($r = 32\text{\AA}$) similar to that of Dextran 20 (M_w^- 22 700). Polymer fragments of HES 350/0.60 excreted 6 to 48 hours after dosing, however, possessed a K_{av} ranging between 0.78 and 0.73 with a Stokes radius ($r = 45\text{\AA}$) similar to that of Dextran 40 (M_w^- 41 000). All filtered polymer fragments were less polydisperse relative to both the injected solution (K_{av} 0.60) and residual material recovered from blood immediately after injection (K_{av} 0.72). These data support the hypothesis that the excretion of HES 350/0.60 occurs in two distinct phases: a rapid phase of elimination dependent on the M_n^- of the injected solution, and a slower phase dependent on the MS (degree of resistance to α -amylase attack). This study, in conjunction with our previous investigation of the changes in circulating HES 350/0.60, define the basic differences between clearance and excretion of the dextrans and of the rapidly degraded species of HES. These data are relevant to the utilisation of HES 350/0.60 during centrifugal leucapheresis.

A variety of species of hydroxyethyl starch (HES) has been developed for use in the management of hypovolaemia and as adjuncts to centrifugal leucapheresis.¹⁻³ The ability to alter the intravascular persistence concomitant with H₂O-binding potential of this glycogen-like amylopectin is primarily a function of the degree of MS on the parent molecule but is also partially dependent on a sub-population of molecules contained in the injected solution and characterised by the M_n^- .⁴ After an initial rapid clearance of colloid from blood after dosing, the major portion of the injected material is hydrolysed to smaller polymer fragments^{5,6} which are subsequently filtered at the glomerulus,^{7,8} and a portion of the total administered dose is taken up by the reticuloendothelial system.⁹ Changes occurring in the molecular-size composition of circulating residual HES remaining in the intravascular space after dosing has been described in detail.^{5,6,10} The size distribution of polymer frag-

ments of HES excreted in urine as a function of time after the injection, however, has not been well characterised. The present study, therefore, is an investigation of the characteristics of the size composition of the polymer fragments excreted over a long-term period of observation in normal man dosed with

Abbreviations:

Molar hydroxyethyl group substitution on the parent amylopectin molecule (MS). This may be calculated as:

$$MS = \frac{W_H}{1 - W_H} \times \frac{162}{44},$$

where W_H is the weight-fraction of hydroxyethyl groups in the polymer.

Number average molecular weight (M_n^-) and weight average molecular weight (M_w^-). These terms may be calculated as:

$$M_n^- = \frac{1/\sum w_i}{M_i} \text{ and } M_w^- = \sum w_i M_i,$$

where w_i is the weight-fraction of the species of molecular weight M_i .

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a new species of HES (HES 350/0-60) possessing an M_w of 350 000 combined with an MS of 0-60. This new species of HES is eliminated from blood at a rate greater than the presently approved long-acting dosage form of HES (HES 450/0-70, M_w 450 000, MS 0-70)⁶ and may therefore be more appropriate for utilisation during centrifugal leucapheresis.

Material and methods

SUBJECTS AND PROCEDURES

The study group comprised five normovolaemic men (aged 20-21) of good health. Their body surface areas (BSA) ranged from 1.80 to 2.01 m², which corresponded approximately to whole blood volumes of 4500 to 5035 ml. The experiment was thoroughly explained to each of the test subjects and their consent was obtained.

A standardised dose (30g/m² BSA) of 6% (w/v) solution of HES 350/0-60* suspended in 0.9% isotonic saline was infused through a 19-gauge cannula inserted in a forearm vein over a 52- to 67-minute period. The average rate of injection was 0.24 ± 0.02 SD ml/kg per min. The methods for the collection of samples of whole blood after injection and the subsequent preparation of sera have been described previously.⁶ Aliquots of urine spontaneously voided by each test subject were collected in the intervals 0-1, 1-3, 3-6, 6-12, 12-24, 24-48, and 48-72 hours after dosing. All samples of collected urine were centrifuged at 3000 *g* (Sorvall RC2-B centrifuge, USA) for 20 minutes at 4°C to remove sediment.

LABORATORY DETERMINATIONS

To 1 ml of sediment-free urine collected at various intervals after dosing was added 3 ml of 30% (w/v) potassium hydroxide, and the subsequent admixture was boiled for 1 hour to destroy monosaccharides. After the sample had been cooled, neutralisation was achieved by the addition of 9 ml of 5% (w/v) sulphuric acid. The total quantity of HES 350/0-60 contained in these prepared samples was then determined in triplicate by means of a simplified anthrone technique.¹¹

GEL FILTRATION

Urine collected from two test subjects (A and B) up to 48 hours after injection was concentrated by ultrafiltration and dialysed in Cellophane tubing (diameter 6.2 mm and pore size < 7.2 nm) to remove contaminating salts. The resulting concentrate was applied to a column of Sepharose CL-4B (Pharmacia

*(M_w 350 000; intrinsic viscosity 0.20 dl/g; MS, 60 hydroxyethyl groups/100 glucose residues)

Fine Chemicals, Sweden) in M/15 sodium phosphate buffer (pH 7.5, one-volume) and 0.9% isotonic saline solution (9-volumes), as previously described.⁵ A total of 50 mg of HES 350/0-60 material contained in either serum (as previously described⁶) or urine was utilised at each interval of sampling. An aliquot of urine (containing 100 mg of HES 350/0-60 material) collected from subject B 1 hour after dosing was ultrafiltered and dialysed (as described above) to a volume of 4 ml. A 2 ml (50 mg) portion of this solution was subjected to gel filtration on a column of Sephadex G-200 (Pharmacia Fine Chemicals, Sweden) under the conditions described above. The remaining 2-ml portion was incubated for 18 hours at 37°C with the α -amylase contained in 1 ml of centrifuged human saliva in the presence of sodium azide preservative. The resultant solution was then subjected to gel filtration on Sephadex G-200. The effluent solution collected from both gel columns was continuously monitored by a differential refractometer (Waters Model R4, USA) operating a chart recorder. Both columns were calibrated with Blue Dextran (M_w 2 000 000) for V_o and sodium iodide for V_t . The Sepharose CL-4B column was further calibrated with both Dextran 20 (M_w 22 7000, M_n 17 000, $M_w/M_n = 1.34$) and Dextran 40 (M_w 41 000, M_n 26 000, $M_w/M_n = 1.58$). The K_{av} for each of the peaks eluted from both columns was calculated according to the formula:

$$K_{av} = \frac{V_e - V_o}{V_t - V_o}$$

Results

RATE AND OVERALL CUMULATIVE EXCRETION OF HES 350/0-60

In five normovolaemic subjects given a standardised injection of 30 g/m² BSA HES 350/0-60, an average of $7.19 \text{ g} \pm 1.93$ SD of this material was excreted in urine 1 hour after injection, which represented the loss of approximately 13% of the total dose administered (Fig. 1). The rate of excretion of HES 350/0-60 diminished thereafter, and by 72 hours after dosing 45% of the initial dose had been voided in urine.

GEL FILTRATION

Sepharose CL-4B

The molecular-size distribution of HES 350/0-60 material excreted in the urine of test subjects A and B up to 1 hour after dosing is displayed in Fig. 2. The molecular composition of HES 350/0-60 material recovered either in serum immediately after injection⁶ or excreted in urine during the first hour after injection is less polydisperse relative to the

Fig. 1 (Above) Intravascular survival, expressed as log %, in five normal men dosed with an average of $56.6 \text{ g} \pm 2.2 \text{ SD}$ of HES 350/0-60.⁸ Each point represents the mean of five determinations. The concentration of HES 350/0-60 measured in serum immediately after injection is expressed as 100%. (Below) Rate of urinary HES 350/0-60 excretion (○) concomitant with the overall cumulative excretion (●) is shown for these same five subjects. Each point represents the mean of five determinations. The average rate of excretion of HES 350/0-60 during the first hour after injection was $7.19 \text{ g/h} \pm 1.93 \text{ SD}$. This initial rate of excretion fell to $2.03 \text{ g/h} \pm 0.22$, 0.85 ± 0.32 , 0.80 ± 0.20 , 0.35 ± 0.12 , 0.13 ± 0.03 , and 0.06 ± 0.01 during the postinfusion intervals 1-3, 3-6, 6-12, 12-24, 24-48, and 48-72 hours, respectively. One hour after injection $12.7\% \pm 1.9 \text{ SD}$ of the total injected dose of HES 350/0-60 appeared in urine. This increased to $19.9\% \pm 0.4$, 24.4 ± 1.0 , 31.3 ± 0.9 , 37.3 ± 0.7 , 42.7 ± 0.8 , and 45.2 ± 0.2 , at 3, 6, 12, 24, 48, and 72 hours after injection respectively.

injected solution. Filtered HES 350/0-60 polymer fragments recovered up to 1 hour after dosing in subjects A and B possessed a Stokes radius ($r = 32\text{\AA}$) similar to that of Dextran 20. Polymer fragments of HES 350/0-60 excreted 6 to 48 hours after injection, however, possessed a K_{av} ranging between 0.78 and 0.73 and having a Stokes radius ($r = 45\text{\AA}$) similar to that of Dextran 40 (Table).

Sephadex G-200

Comparison of the two elution profiles (Fig. 3) reveals that HES 350/0-60 polymer fragments excreted during the first hour after dosing had not been hydrolysed by serum α -amylase to the maximal possible extent, since subsequent incubation with α -amylase contained in human saliva caused further production of less polydisperse material. This was witnessed by the shift of molecular size distribution

Fig. 2 Gel filtration on a column of Sepharose CL-48 (details in Methods) of 50 mg of HES 350/0-60 (—) of M_w 350 000 and a similar quantity of HES 350/0-60 recovered from: (1) the serum (10 mg of material was pooled from each of five test subjects dosed with 30 g/m^2 BSA) immediately after injection (●—●),⁸ and (2) the urine of two of the above test subjects (A ■—■, B △—△) 1 hour after dosing. The column was calibrated by use of 50 mg of Dextran 20 (M_w 22 7000); the peak of elution corresponded to K_{av} 0.83 (----). The infused solution of HES 350/0-60 showed a peak with K_{av} 0.60. Residual material recovered from serum possessed a K_{av} of 0.72. HES 350/0-60 material excreted in the urine of subjects A and B yielded a K_{av} of 0.78-0.73 (see Table for actual values). Polymer fragments of HES 350/0-60 filtered through the glomerulus up to 1 hour after dosing possessed a Stokes' radius of $32\text{\AA}$ similar to that of Dextran 20,¹⁶ while material excreted 6 to 48 hours after injection had a Stokes' radius of $45\text{\AA}$ similar to that of Dextran 40.¹⁶

K_{av} values for elution peaks obtained after separation of various molecular weight HES 350/0-60 material on a column of Sepharose CL-48 (see Methods)

	Observation period after injection (hours)		
	6-12	12-24	24-48
K_{av} :			
Subject A	0.78	0.76	0.75
Subject B	0.76	0.75	0.73

The peaks were obtained from aliquots of urine collected from subjects A and B 6-48 hours after dosing. As a reference standard, dextran 40 (M_w 41 000) with a Stokes' radius of $45\text{\AA}$ ¹⁶ was separated on the same column of Sepharose CL-48 with a peak corresponding to K_{av} 0.76.

toward the low-molecular-weight region of the column.

Discussion

The principal reason underlying the development of HES 350/0-60 was the need to provide a greater rate of colloid clearance from blood than is presently available with the approved long-acting dosage form of HES 450/0-70. Recent concern has been raised about the long-term persistence of HES 450/0-70 during centrifugal leucapheresis, as residual colloid

Fig. 3 Sephadex G-200 elution profiles generated from 50 mg of HES 350/0-60 material recovered in the urine (●—●) of subject B (see Fig. 2) up to 1 hour after receiving a dose of 54 g of this substance and 50 mg from the same samples of urine after incubation with human saliva (△—△) for 18 hours at 37°C. The HES 350/0-60 excreted in urine showed a peak with a K_{av} of 0.60, while the same material incubated with saliva displayed a peak with a K_{av} of 0.80. These data reveal that HES 350/0-60 recovered from urine during the rapid phase of excretion is capable of still further catabolism by the action of α -amylase contained in saliva.

can be detected in blood 17 weeks after dosing.^{10 12} As the usefulness of HES 450/0-70 in decreasing the suspension stability of blood would in most cases be restricted to a period of 2 to 4 hours during the collection of leucocytes, residual colloid remaining in blood after this interval appears to serve no practical purpose. By reducing both the M_w and MS of HES 450/0-70, we had previously demonstrated⁶ that a reasonable proportion of the initial peak dose of HES 350/0-60 remains 6 hours after injection, but, more importantly, up to 192 hours after dosing this new species of HES is removed from the intravascular space approximately twice as rapidly as HES 450/0-70.⁶ The greater rate of clearance of HES 350/0-60 from blood appears to be related to both the 'pattern' and 'amount' of hydroxyethylation incorporated into the parent amylopectin molecule. Effective, sustained retardation of attack of α -amylase implies that attachment of the hydroxyethyl group on any glucose residue within a specific five-unit amylose substrate must deviate from acceptable patterns of substitution.¹³ As the MS is increased from 0.7 to 2.0, there is a greater likelihood that unfavourable substitutions will occur.¹⁴ At these degrees of MS, the frequency of di-, tri-, and tetra-substituted glucose residues is significantly higher. Low degrees of MS (0.43-0.60) probably increase the likelihood that *only* mono-substitution occurs on individual glucose residues, which facilitates degradation by α -amylase. As a result, a greater rate of clearance is achieved.^{5 6}

Residual HES material remaining in the intravascular space after dosing is either catabolised by

α -amylase to less polydisperse fractions^{5 6 10} capable of being filtered at the glomerulus or taken up by the reticuloendothelial system.⁹ The overall persistence of any species of HES is dependent on the MS and is somewhat independent of the M_w of the injected solution.⁴ During the initial hour after dosing, however, the total amount of colloid appearing in urine is an inverse property of a sub-population of small molecules contained in the injected solution and characterised by the M_n .⁴ The changes in the molecular size distribution of HES 350/0-60 material recovered either in blood immediately after injection (K_{av} 0.72) or voided in urine (K_{av} 0.88-0.84) 1 hour after dosing, relative to the highly disperse injected solution (K_{av} 0.60), clearly demonstrates the rapid removal of these low-molecular-weight polymers (Fig. 2). These small polymer fragments possess a molecular weight below that of the limit of glomerular filtration and appear to be little affected by the action of α -amylase, which is clearly evident from the further change in the molecular-size distribution after incubation with human saliva (Fig. 3). After this phase of rapid clearance from blood (and subsequent appearance in voided urine), however, the rate of colloid excretion is an inverse property of the MS of the injected solution.^{2 4 7} The data thus obtained with HES 350/0-60 further substantiate the hypothesis that the rate of polymer excretion for any given species of HES is influenced by the MS of the injected solution.

The characteristics of the molecular size composition of excreted HES 350/0-60 polymer fragments is also of interest. Previously it was shown that polymer fragments of HES 264/0-43 recovered up to 12 hours after dosing displayed a peak of elution with K_{av} 0.83 when separated on a column of Sepharose CL-4B.¹⁵ A similar value of K_{av} (0.88-0.84) was obtained in the present study with HES 350/0-60 material recovered from urine only up to the first hour after dosing (Fig. 2). In the interval 6 to 48 hours after injection, the recovered HES 350/0-60 polymer fragments possessed a K_{av} ranging between 0.78 and 0.73 (Table), which appears to indicate that the limit of glomerular filtration for excretion of HES 350/0-60 polymer fragments is similar to that of a dextran possessing a M_w of 41 000 with a Stokes radius of 45Å.¹⁶ Thus, the rate at which polymer fragments of HES 350/0-60 are excreted appears to depend on attainment of a specific molecular weight (or molecular radius) below the threshold of glomerular filtration.

The results of the present investigation of the excretion kinetics of HES 350/0-60, when considered with our previous work on the molecular size distribution changes occurring in the blood of these same subjects,⁶ clearly defines, for the first time, the

differences between clearance and excretion of the dextrans and of HES. Basically, as a function of time after infusion, the molecular weight of dextran polymers remaining in the intravascular space¹⁷ or excreted in urine¹⁸ become *larger* rather than *smaller*. This change in the character of material remaining in blood is the result of a disappearance of the smaller molecules into the extravascular space and across the glomerular membrane into the urine. The urinary excretion of dextran undergoes a similar shift in its molecular weight composition, which reflects that occurring in blood.¹⁸ With the rapidly catabolised species of HES (species of HES with MS of 0.43-0.60) the material remaining in blood becomes *smaller* with time after infusion.^{5,6} The molecular-size composition of HES 350/0-60 polymer fragments excreted 6 to 48 hours after dosing is of a relatively constant size (Table). (The elution profiles seem to indicate the excretion of successively larger molecules, but we believe these changes to be slight.) Thus these differences in serum persistence and urinary excretion between the dextrans and the rapidly catabolised species of HES reflect two different mechanisms of action.

In conclusion, the rate at which polymer fragments of HES 350/0-60 are excreted in urine appears to be related to the attainment of a specific molecular weight (or molecular radius) in blood (below the glomerular threshold), which in turn is dependent on the MS of the injected solution. The characteristics of the molecular-size composition of excreted polymer fragments appear to differ from those of the dextrans, and the different mechanism by which these fragments are cleared is especially evident in relation to the changes occurring in the residual material remaining in blood. The point to be stressed in this context is that hydroxyethylation has a greater influence on appearance of polymer fragments in urine than does molecular weight, which again underlines the basic difference between the dextrans and HES. These data should be relevant to normal donors from whom leucocytes are removed by centrifugal blood cell separators.

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Requests for reprints to: Dr John Milton Mishler, IV, Chief, Blood Resources Branch, Division of Blood Diseases and Resources, NHLBI, NIH, 7550 Wisconsin Avenue, Bethesda, Maryland 20205, USA.