Br. J. Pharmac. (1970), **38**, 725–730.

Changes in the molecular composition of circulating hydroxyethyl starch

S. P. FARROW, M. HALL AND C. R. RICKETTS

M.R.C. Industrial Injuries and Burns Research Unit, Birmingham Accident Hospital, Bath Row, Birmingham 15

Summary

1. Hydroxyethyl starch (HES) of molecular weight 450,000 has been extensively investigated as a plasma volume expander and HES of molecular weight 60,000 has been proposed for use as a cryoprotective agent in the freezing for preservation of red blood cells. In these applications considerable amounts of HES may be injected intravenously and it is therefore desirable to understand its behaviour *in vivo*.

2. After intravenous injection into rabbits, plasma levels of HES and clinical dextrans were determined using anthrone. HES persisted longer in the circulation than did the dextrans.

3. HES was recovered from the bloodstream of rabbits and shown by gel filtration to be of a narrower molecular size distribution than the injected material. Smaller molecules were removed, presumably by filtration at the glomerulus. Larger molecules may have been removed by cells of the reticulo-endothelial system or possibly through the action of amylase.

4. The amylase in saliva was shown to break down HES into molecules of intermediate size with very little production of maltose units.

Introduction

Hydroxyethyl starch (HES) has been extensively investigated as a plasma volume expander and there are now several reports of its clinical use (Ballinger, Murray & Morse, 1966; Lee, Cooper, Weidner & Murner, 1968; Solanke, 1968; Gollub, Schechter, Hirose & Bailey, 1969). At present dextran is the most widely used plasma volume expander. Comparison of intrinsic viscosity and molecular weight data (Table 1) shows that for a given molecular weight HES has a much lower intrinsic viscosity than dextran (compare Greenwood & Hourston, 1967) and probably therefore a more compact molecular shape. Elongated molecules such as dextran and some plasma globulins cause an increased sedimentation rate of red cells when they are present in blood at relatively high concentrations. Tests similar to those used for dextran (Ricketts, 1966) showed that for a given molecular weight and concentration HES caused a lower rate of red cell sedimentation than dextran. It therefore seemed worth while knowing more about the behaviour of HES *in vivo* whatever the relative merits of HES and dextran as plasma volume expanders might eventually prove to be.

Polyvinyl pyrollidone protects red cells against the effects of freezing, and recently the theoretical basis of this colloid effect has been considered in relation to the established action of glycerol (Farrant, 1969). Hydroxyethyl starch also protects red cells against the effects of freezing (Knorpp, Merchant, Gikas, Spencer & Thompson, 1967), but apparently dextran is not nearly so effective. When the thawed red cells are subsequently transfused these colloids may enter the circulation of the recipient. The concentrations used are rather high, about 12%, so that the amount of HES infused may be quite large depending on whether or not the red cells are washed and on the amount of blood given. Because it is slowly broken down by enzymes in the body, HES may be more acceptable than polyvinyl pyrollidone. The use of HES as a cryoprotective substance for red cells is thus an additional reason for exploring its behaviour *in vivo*.

Methods

Hydroxyethyl starch (McGaw Laboratories, Glendale, California) intended for use as a plasma volume expander, Lot No. S 3334A, molecular weight 450,000, inherent viscosity* 0·25 dl/g, degree of substitution 0·7, concentration 6%, and hydroxyethyl starch intended for cryoprotection of red cells, Lot No. A 32, molecular weight 60,000, inherent viscosity 0·18 dl/g, degree of substitution 0·7, concentration 40%, were used in the experiments described; both were in 0·9% sodium chloride solution. A further lot of each type of HES was examined by gel filtration on Sepharose 4B; no important difference from the material used in these experiments was found. Dextrans 40, 70 and 110 are clinical dextrans of molecular weights approximately 40,000, 70,000 and 110,000, respectively. Other dextrans are fractions with specified molecular weights.

Gel filtration was done on a column of Sephadex G-200 (4·9 cm^2 by 45 cm long) or on a column of Sepharose 4B (4·0 cm^2 by 60 cm long) in M/15 sodium phosphate buffer pH 7·5 (1 volume) plus 0·9% sodium chloride solution (9 volumes). The elution rate was 11–12 ml/h and 5 ml fractions were collected as required. The effluent solution was monitored by a differential refractometer (Waters Model R4) operating a chart recorder. Experiments with solutions of known concentration showed that the recorder readings were linear with concentration. The columns were calibrated with Blue Dextran for void volume (V_o) and sodium iodide for total volume (V_t).

* Inherent viscosity is defined as the limit when C approaches zero of $\frac{2{\cdot}3 \log_{10} \eta_{rel}}{C}$ where η_{rel} is the relative viscosity of the polymer solution and C is the concentration in g/100 ml.

TABLE 1. *Molecular weight and intrinsic viscosity of HES and dextran*

Colloid	M_w	M_n	M_w/M_n	$[\eta]$
Dextran	518,000	199,000	2·6	0·53
HES	450,000	65,000	6·9	0·25
Dextran	370,000	185,000	2·0	0·5
Dextran	75,000	46,000	1·6	0·26
HES	61,800	22,000	2·8	0·15

M_w, weight average molecular weight; M_n, number average molecular weight; $[\eta]$, intrinsic viscosity. The ratio M_w/M_n indicates the width of the dispersion of molecular weight in a polydisperse material.

Experiments in rabbits. 30 ml of a 6% solution of hydroxyethyl starch in 0·9% sodium chloride was injected intravenously into Sandy Lop/New Zealand white cross-bred rabbits of either sex weighing 3 kg. Blood samples with EDTA anticoagulant were taken at intervals and the plasma was separated. After precipitation of the plasma proteins with trichloroacetic acid, total carbohydrate was determined by the anthrone method (Davies, Ricketts & Williams, 1963). In experiments to determine the molecular size distribution of HES remaining in the bloodstream, the trichloroacetic acid filtrate was concentrated by ultrafiltration in Cellophane tubing and the concentrate was applied to the gel filtration column.

Incubation with amylase. Plasma expander HES 6% solution was diluted with an equal volume of phosphate buffer saline containing 0·05% sodium azide. Two ml of centrifuged saliva was added to 18 ml of the solution and the mixture was incubated at 37° C; samples were withdrawn at intervals for viscosity measurements and gel filtration.

Results

Plasma levels. From each determination of total carbohydrate the carbohydrate present in plasma before the injection, 142 ± 14 mg/100 ml, was subtracted so as to obtain as nearly as possible the correct value for concentration of HES in plasma. Ten minutes after injection of 30 ml of 6% HES, the plasma concentration was

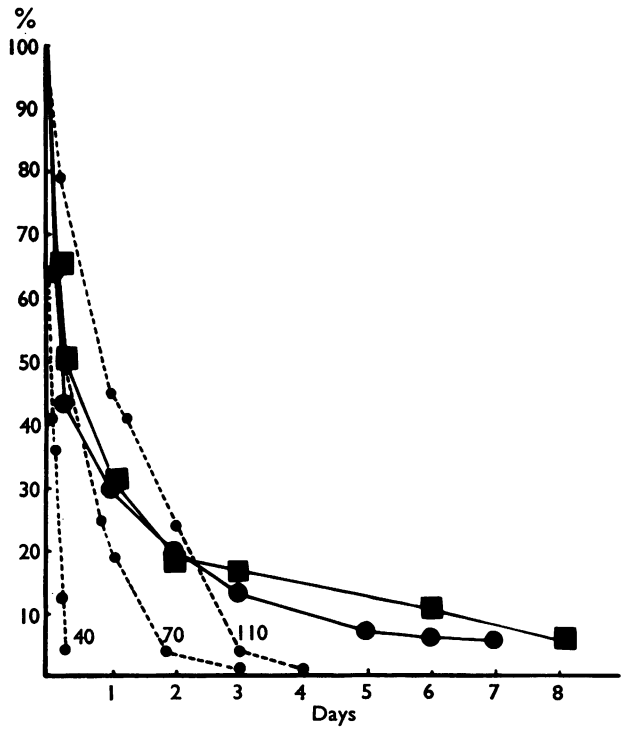

FIG. 1. Plasma concentrations of HES (molecular weight 450,000, ■; molecular weight 60,000, ●) and dextrans (molecular weights 40,000, 70,000 and 110,000 respectively) in rabbits given 30 ml of 6% solution in saline. The concentration is plotted as a percentage of the value 10 min after injection; each curve is the mean for two rabbits. The broken lines show plasma concentrations of dextrans 40, 70 and 110 respectively for comparison.

1·1% (mean of four rabbits). Subsequent values were expressed as a percentage of the 10 min value for each rabbit. Figure 1 shows the fall in plasma level on the succeeding days ; each curve is the mean for two rabbits. Corresponding curves determined in the same way for dextrans 40, 70 and 110 are shown for comparison.

Molecular size distribution. Figure 2 illustrates the molecular size distribution of the HES of molecular weight 450,000 injected into a rabbit compared with the

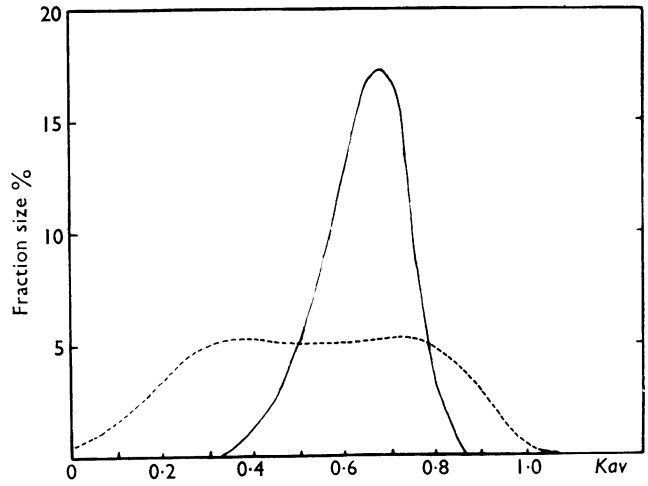

FIG. 2. Gel filtration on a column of Sepharose 4B (details in text) of 50 mg of HES of molecular weight 450,000 (broken line) and a similar amount of HES recovered from rabbit plasma 24 h after injection (solid line). The distribution of molecular size present in the circulation at 24 h is narrower than was present in the HES solution injected. ($Kav=\frac{V_e-V_o}{V_t-V_o}$ where V_e is the elution volume, V_o is the void volume and V_t is the total volume of the gel filtration column.)

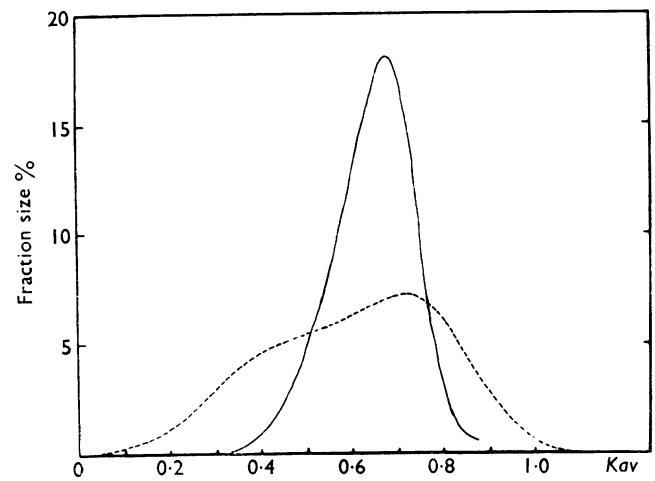

FIG. 3. Gel filtration on a column of Sepharose 4B of 50 mg of HES of molecular weight 60,000 (broken line) and a similar amount of HES recovered from rabbit plasma 24 h after injection (solid line). Again the distribution of molecular size present in the circulation 24 h after injection is narrower than was present in the HES solution injected. ($Kav=\frac{V_e-V_o}{V_t-V_o}$ where V_e is the elution volume, V_o is the void volume and V_t is the total volume of the gel filtration column.)

size distribution of molecules remaining in the circulation 24 h later. The differences between the two curves show that a narrower distribution in the middle of the range remains in the circulation. At 6 days, when the concentration was much lower, HES was again recovered from the circulation of the same rabbit. It was found that the distribution had shifted slightly (0·05 *Kav**) toward the high molecular weight end of the range. Fractions corresponding with the peaks of material recovered from the circulation were combined and concentrated by ultrafiltration ; the presence of carbohydrate in approximately the expected amount was confirmed by colorimetric determinations with anthrone. As a control experiment plasma from a rabbit not given HES was treated with trichloroacetic acid and submitted to gel filtration in the same way ; at the position of the HES peak, *Kav* 0·725, no material was detected. Figure 3 shows similar information for HES of molecular weight 60,000.

Incubation with amylase. Sephadex G-200 is not capable of fully resolving the molecular size range present in either sample of HES, and much of the sample eluted from a column is present in the void volume. However, the action of amylases would be expected to produce fragments of the original HES molecules resolvable on Sephadex G-200. Figure 4 shows the elution curve for HES of molecular weight 450,000 before incubation with amylase and samples taken after 20 and 43 h of incubation. At 20 h it is apparent that much of the material originally appearing in the void volume has been broken down to molecules resolvable on Sephadex G-200 and this breakdown is still continuing at 43 h. There was a concomitant fall in relative viscosity of the solution from 1·48 to 1·28 in 17 h. Only a very small increase in reducing power could be detected in tests with Fehling's solution, showing that there was very little production of maltose units.

Discussion

In the first 12 h after injection, the fall in plasma concentration of HES was similar to that of dextran 70. Subsequently more HES than dextran remained in

* $Kav = \frac{V_e - V_o}{V_t - V_o}$ where V_e is the elution volume, V_o is the void volume and V_t is the total volume of the gel filtration column.

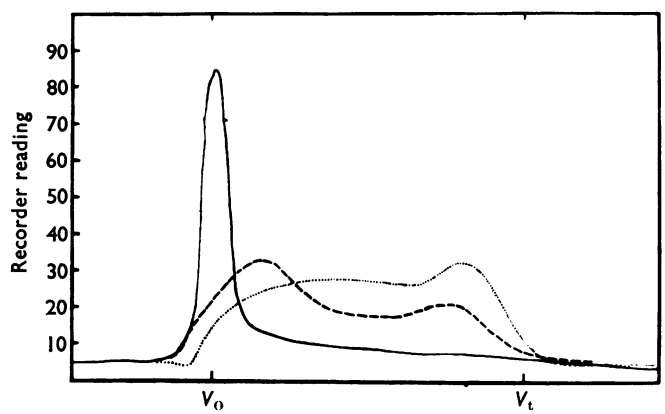

FIG. 4. Gel filtration on a column of Sephadex G-200 (details in text) of 25 mg quantities of HES of molecular weight 450,000 (solid line) and the same HES incubated with salivary amylase for 20 h (dashed line) and 43 h (dotted line). Note that the high molecular weight material appearing in the void volume (V_0) has been broken down by amylase to medium sized molecules.

the circulation, more even than of the higher molecular weight dextran 110. This is so for both samples of HES with molecular weights of 450,000 and 60,000. The curves for molecular size distribution in Figs. 2 and 3 show that there is not as much difference between the two samples as their average molecular weights would suggest; both samples have in fact an extraordinarily wide molecular size distribution. The distribution of molecular size in HES recovered from the circulation 24 h after injection was much narrower than the distribution in the HES originally injected. Smaller molecules had been removed, presumably by filtration at the glomerulus. Larger molecules had been removed possibly by uptake into cells of the reticulo-endothelial system or possibly through the action of amylase enzymes in reducing their molecular size. It was interesting that the same size distribution was found in the circulation whichever sample of HES had been injected; possibly this distribution would be the optimum for intravenous use for maintenance of plasma volume. The action of amylase was examined using salivary amylase and it is clear from the curves for molecular size distribution obtained by gel filtration on Sephadex G-200 that the large molecules are broken down to medium sized molecules with the production of very small amounts of maltose or hydroxyethyl maltose. Evidently the degree of substitution 0·7 is not sufficient to render the molecules completely resistant to amylase. While resistance to amylase may be desirable in a plasma volume expander, a cryoprotective substance should disappear completely from the body within a short time and a lower degree of substitution than 0·7 may be advantageous for this purpose. The distribution of hydroxyethyl groups among molecules is probably not uniform and is probably determined by statistical considerations. Those molecules remaining in the circulation for a prolonged time are likely to be those with the highest degree of substitution and greatest resistance to hydrolysis by amylase. Thompson & Walton (1963) have claimed, however, that tissue storage of HES in the major viscera of dogs was distinctly less than that of dextran.

REFERENCES

BALLINGER, W. F., MURRAY, G. F. & MORSE, E. E. (1966). Preliminary report on the use of hydroxyethyl starch solution in man. *J. surg. Res.*, **6**, 180–183.

DAVIES, J. W. L., RICKETTS, C. R. & WILLIAMS, B. N. (1963). Plasma volume expansion by rapid infusion of a low molecular weight dextran. *Br. J. Pharmac. Chemother.*, **21**, 220–225.

FARRANT, J. (1969). Is there a common mechanism of protection of living cells by polyvinyl pyrrolidone and glycerol during freezing? *Nature, Lond.*, **222**, 1175–1176.

GOLLUB, S. G., SCHECHTER, D. C., HIROSE, T. & BAILEY, C. P. (1969). Use of hydroxyethyl starch solution in extensive surgical operations. *Surg. Gynec. Obstet.*, **128**, 725–728.

GREENWOOD, C. T. & HOURSTON, D. J. (1967). Physicochemical studies on starches. Part 33, Some physicochemical properties of hydroxyethyl starch used as a volume-extender for blood plasma. *Die Stärke*, **19**, 243–246.

KNORPP, C. T., MERCHANT, W. R., GIKAS, P. W., SPENCER, H. H. & THOMPSON, N. W. (1967). Hydroxyethyl starch: extracellular cryoprotective agent for erythrocytes. *Science, N.Y.*, **157**, 1312–1313.

LEE, W. H., COOPER, N., WEIDNER, M. G. & MURNER, E. S. (1968). Clinical evaluation of a new plasma expander, hydroxyethyl starch. *J. Trauma*, **8**, 381–393.

RICKETTS, C. R. (1966). Molecular composition of transfusion dextran. *Br. med. J.*, **2**, 1423–1426.

SOLANKE, T. F. (1968). Clinical trial of 6% hydroxyethyl starch (a new plasma expander). *Br. med. J.*, **3**, 783–785.

THOMPSON, W. L. & WALTON, R. P. (1963). Blood changes, renal function and tissue storage following massive infusion of hydroxyethyl starches. *Fedn Proc.*, **22**, 640, Abstr. No. 2869.

(*Received November* 28, 1969)