RETENTION AND STORAGE SITES OF RADIOACTIVE POLYVINYLPYRROLIDONE

B. Hulme,* P. W. Dykes, I. Appleyard and D. W. Arkell

University of Birmingham, Birmingham, England

Polyvinylpyrrolidone (PVP) was first introduced in Germany (*1*) as a nontoxic plasma substitute; during and after the Second World War it was administered to over 500,000 patients (*2*) without adverse reactions although histopathological studies in man (*3*) and experimental animals (*4*) demonstrated phagocytosis of the polymer by macrophages for several weeks after its infusion. Forty to 60% of the PVP was excreted in the urine, mainly during the initial post-infusion period, and no other significant site of excretion was found. The use of PVP as a plasma substitute rapidly declined with the introduction of transfusion Dextran; although this material was stored in the reticuloendothelial system, it was capable of slow degradation to glucose.

PVP labeled with ^{131}I was introduced by Gordon (*5*) as a simple way of measuring gastrointestinal permeability to macromolecules in patients with a suspected protein-losing enteropathy; the labeled polymer has been given in an attempt to localize brain lesions (*6*) and has been used to measure glomerular permeability in patients with and without renal disease (*7*). We have determined the quantity in storage sites of injected radioactively labeled PVP because it has been suggested (*8*) that the material is selectively stored in the liver and that the irradiation to this organ could be high enough to be hazardous.

MATERIALS AND METHODS

Unfractionated ^{131}I PVP (preparation A_1 with an average molecular weight of 38,000) and two fractionated preparations (A_2 and A_3, with average molecular weights of 32,000 and 12,500, respectively) were supplied as sterile isotonic pyrogen-free solutions by the Radiochemical Centre, Amersham. Three relatively monodispersed fractions were obtained by gel filtration of the A_1 material on a Sephadex-G.200 70 $\times$ 2-cm column in the laboratory; 10-ml fractions of the eluate were collected, and single fractions—S_1 with an average molecular weight of 100,000, S_2 with an average molecular weight of 30,000 and S_3 with an average molecular weight of 26,000—were used in the experiments.

The molecular-size distribution of the six polymer preparations and samples of human and rat urine obtained after parenteral administration of the A_1 fractions was determined by gel filtration on Sephadex-G.200 columns 70 cm long and 2 cm in diameter by eluting them with 0.15 *M* sodium chloride and collecting 2-ml fractions of the eluate. To compare elution patterns of different columns, the results were expressed as elution coefficients K_{av} (*9*) where $K_{av} = V_e - V_o/V_t - V_o$, V_o = void volume of the column, V_t = total volume of the column and V_e = elution volume of the PVP.

Human subjects received 180 mg of potassium iodide daily before and for 8 days after the intravenous injection of known amounts of the A_1 material in eight patients and the A_2 fraction in three further patients. Complete 24-hr urine and fecal collections were made for the subsequent 4 days, and in two subjects serial venous blood samples were obtained; 10-ml aliquots of urine, homogenized feces, plasma and appropriate standard solutions were counted in a scintillation well counter and scaler to an error of less than 3%.

Single intraperitoneal injections of known amounts of the different polymer fractions were given to mice and rats using the scheme in Table 1. The rate of excretion of the labeled material was monitored by whole-body counting using a single sodium iodide crystal, standard counting electronics and a sufficient amount of lead shielding; the animals were held in

TABLE 1. DISTRIBUTION AND NUMBER OF ANIMAL SPECIES RECEIVING PVP FRACTIONS

	^{131}I PVP preparations					
Species	A_1	A_2	A_3	S_1	S_2	S_3
Man	8	3				
Mice				2	3	3
Rats	2	2	2			

Received Aug. 24, 1966; revision accepted Aug. 2, 1967.

* Present address: St. Mary's Hospital, London.

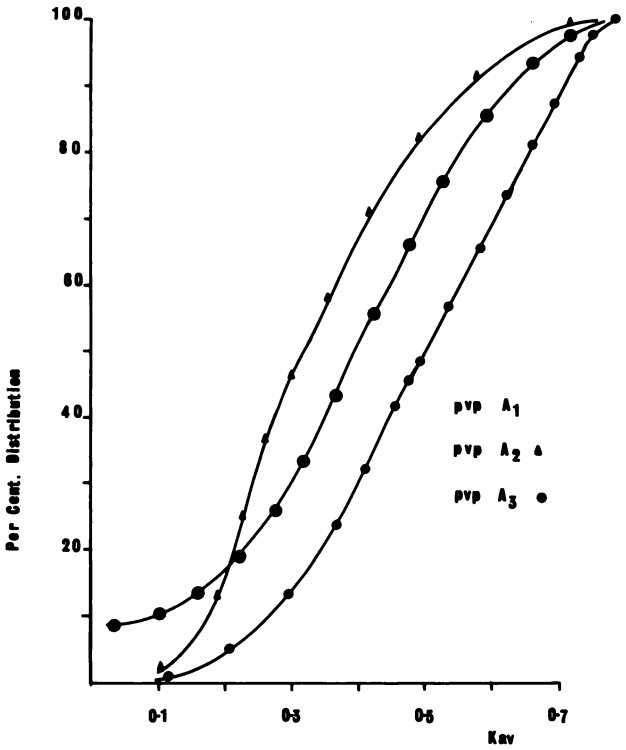

FIG. 1. Molecular-size distribution of PVP fractions A_1, A_2 and A_3.

a plastic box during counting, and the same geometrical locations were used on each occasion. The rats were killed 5 days after PVP injection while the mice were killed at intervals up to 29 days after injection; organs were removed, weighed and counted in the scintillation well counter and the tissue activity was calculated.

RESULTS

The molecular-size distribution of the fractions A_1, A_2 and A_3 were determined by gel filtration on Sephadex G.200 and are shown in Fig. 1; the preparations are polydispersed with a wide range of molecular sizes, and the fractionation procedure has reduced the proportion of higher-molecular-weight material. Figure 2 shows molecular-size distribution of the S_1, S_2 and S_3 preparations; the range of molecular sizes in each fraction is less than that of the Radiochemical Centre material.

The quantity of ^{131}I PVP retained by the different species is shown in Table 2; over 50% of the A_1 preparation remained in human subjects, and values of 40% were obtained for the lower-molecular-weight A_2 fraction. Fecal excretion of the A_1 and A_2 preparations were identical with mean values of 0.7 and 0.8% of the administered dose, respectively. The greater renal excretion of the lower molecular fraction was marked during the first 24 hr after injection, and no significant difference was observed during the subsequent days (Fig. 3). Rats excreted a larger proportion of each PVP fraction (Table 2), suggesting a species difference in glomerular permeability similar to that demonstrated by Wallenius (*10*) for Dextran. Technical difficulties in collecting accurately timed urine samples from rats prevented formal renal clearances, but gel filtration of rat and human urine following administration of fraction A_1 showed higher-molecular-weight material in the rat urine (Fig. 4).

TABLE 2. PERCENTAGE RETENTION OF ^{131}I-PVP 5 DAYS AFTER ADMINISTRATION

Species	PVP preparation					
	A_1	A_2	A_3	S_1	S_2	S_3
Man	55 51					
	55 58	42				
	57 59	42				
	50 55	34				
Mice				77	14	2.5
				78	14	2.5
					14	2.1
Rats	44	38	12			
	43	37	13			

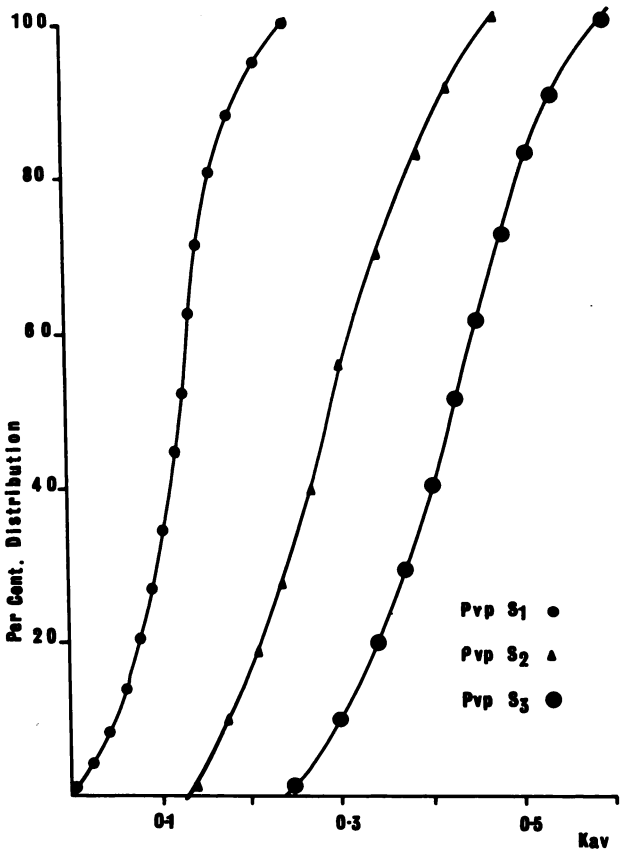

FIG. 2. Molecular-size distribution of PVP fractions S_1, S_2 and S_3.

The retention of the narrow molecular-weight-range S_1, S_2 and S_3 preparations in mice confirmed that the excretion of ^{131}I PVP was inversely proportional to the average molecular weight of the polymer (Table 2).

The plasma radioactivity showed an exponential decay over the 100 hr in which it was measured in two humans following the intravenous injection of the A_1 material. The results obtained from one subject are shown in Fig. 5, and less than 1% of the original radioactivity remained in the plasma although only 45% of the material had been excreted in urine and feces.

The distribution of ^{131}I-PVP fractions in rat tissues 5 days after a single intraperitoneal injection of polymer is shown in Table 3. The material appeared to be retained in the liver and spleen; the relative proportions of each fraction in the tissues were similar apart from a disproportionate increase in kidney radioactivity of the low-molecular-weight A_3 fraction.

Tissue specific activities of mice which were killed at intervals up to 29 days after a single intraperitoneal injection of the S_1, S_2 or S_3 preparations are shown in Table 4; animals that had received the lowest-molecular-weight material showed localization of radioactivity to the kidney. The higher-molecular-weight fractions were selectively stored in the liver, spleen, thymus and lymph node; the retention in these organs increased with time and was more marked for the spleen; the concentration of the S_1 material in this organ was 22 times greater than that for muscle. The tissue distribution of PVP did not depend on the vascularity of the organs because very low plasma radioactivity—less than that

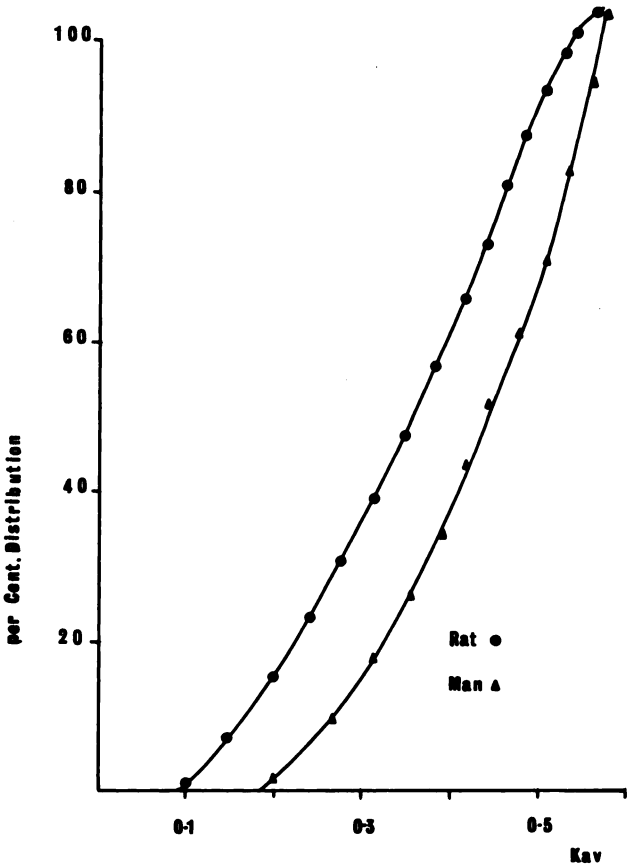

FIG. 4. Molecular-size distribution of A_1 fractions excreted in human and rat urine, determined by Sephadex G.200.

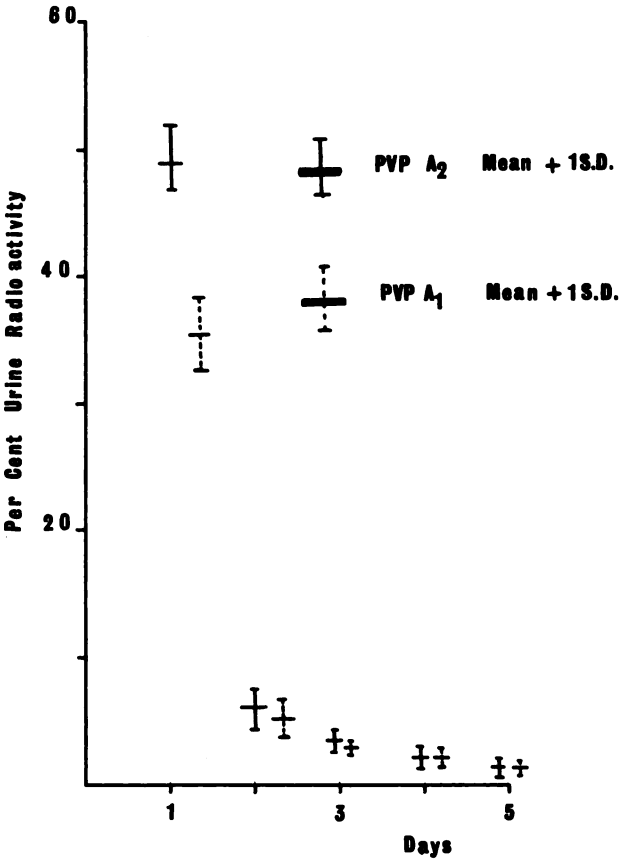

FIG. 3. Rate of renal excretion of different molecular-weight PVP fractions in man.

TABLE 3. MEAN DISTRIBUTION OF ^{131}I-PVP IN RATS AS PERCENTAGE OF ADMINISTERED MATERIAL 5 DAYS AFTER INTRAPERITONEAL INJECTION

	PVP preparation		
Organ	A_1	A_2	A_3
Whole body	43	37	13
Liver	4.10	4.20	1.04
Spleen	1.70	1.10	0.25
Kidney	0.28	0.22	0.35
Lung	0.55	0.1	0.01
Heart	0.11	0.10	0.04

TABLE 4. RELATIVE TISSUE CONCENTRATIONS OF ^{131}I-PVP FRACTIONS IN MICE KILLED AT DIFFERENT TIMES AFTER ADMINISTRATON

	Ratio of ^{131}I PVP fraction*				
	S_1		S_2		S_3
	2 days	29 days	2 days	6 days	2 days
Spleen	5.1	21.8	1.3	5.7	1
Liver	2.3	6.9	1.6	3.8	1
Lymph node	3.4	4.0	1	—	1
Thymus	2.8	3.7	1.6	4.6	1
Kidney	2.2	—	1	—	2.1
Bone	1	1	1	1	1
Muscle	1	1	1	1	1

* (cps/gm tissue)/cps/gm muscle).

for skeletal muscle—was found in all animals at death. Furthermore a rat was killed 1 hr after receiving an intravenous injection of the A_1 material, and the distribution of radioactivity in the tissues is shown in Table 5; although a high proportion of polymer was found in the liver, the results for other organs were dissimilar from those found in animals killed later.

DISCUSSION

The renal excretion of low-molecular-weight PVP is rapid with maximal loss occurring during the first 24 hr after administration. The differences between fractions A_1 and A_2 can be explained by the rapid renal excretion of the greater proportion of low-molecular-weight polymer in the A_2 fraction during the initial 24 hr. Previous studies (*7*) suggested that this excretion was by glomerular filtration without significant tubular secretion or re-absorption; minimal tubular re-absorption probably does take place as the kidney concentration of the low-molecular-weight-fractions A_3 and S_3 are increased (Tables 3 and 4). Gel filtration on Sephadex G.200 of rat and human urine following administration of the same ^{131}I-PVP preparation shows that a polymer with an equivalent molecular size to K_{av} 0.20 can pass through the glomerular filter. The molecular-size distribution of polymer in rat and human urine suggests that larger-molecular-weight PVP fractions can pass through the rat glomerular filter, confirming the observations of Wallenius (*10*) and perhaps explaining the constant finding of proteinuria in this species.

TABLE 5. RADIOACTIVE DISTRIBUTION IN RAT 45 MIN AFTER INTRAVENOUS INJECTION OF ^{131}I-PVP FRACTION A_1

Organ	Percentage administered ^{131}I-PVP
Liver	9.6
Spleen	0.8
Kidney	0.5
Lung	0.8

Human and animal experiments may not be directly comparable because of the different parenteral administration routes. The greater excretion of the same preparation in the rat following an intraperitoneal injection suggests that the polymer was not retained within the abdominal cavity; direct puncture of the gastrointestinal tract was considered unlikely because a significant proportion of the material remained in the body; the effect of different routes of PVP administration in the same species was not investigated.

The higher-molecular-weight PVP is rapidly eliminated from the blood with a biological half-life of 12 hr. Less than 1% of the original activity is present 100 hr after an intravenous injection, confirming the observations of Tothill (*8*) and Rootvelt (*11*). The material is phagocytized and stored in the reticuloendothelial system in the rat and mouse with greater localization to the liver and spleen. Ravin *et al* (*12*) using ^{14}C and ^{131}I-labeled PVP had previously shown that this localization depended on the molecular weight of the polymer; there was a disproportionate localization to the spleen with time for material with a molecular weight greater than 100,000. The studies with relatively monodispersed S_1, S_2 and S_3 fractions in mice confirmed these observations; there was no specific localization to the liver and spleen of the lowest-molecular-weight S_3

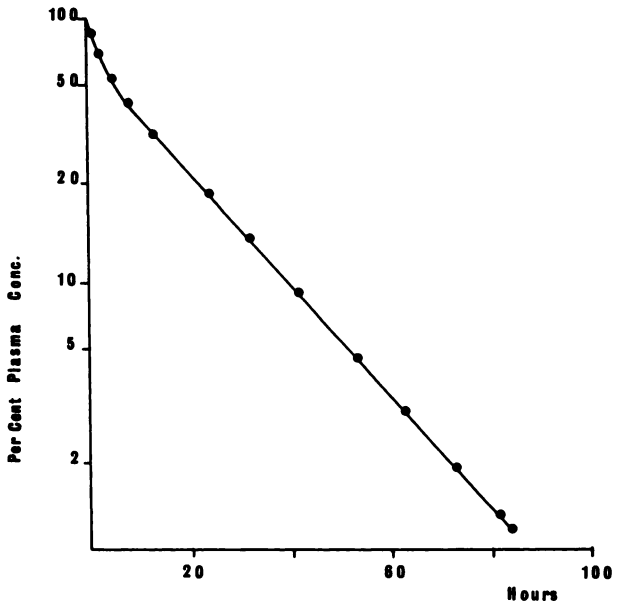

FIG. 5. Elimination of A_1 ^{131}I PVP from plasma in one patient.

fraction. Similar results were obtained 2 days after the administration of the S_2 fraction, but at 6 days the radioactvity in the liver and spleen had increased with greater concentrations in the latter organ (Table 4). The localization to the reticuloendothelial system with a disproportionate increase in spleen radioactivity was demonstrated in the animals that had received the S_1 fraction and were killed 29 days later; the distribution could not be explained by assuming that all the radioactivity in the organs depended on the intravascular radioactivity pool that was significant by this period of time.

Tothill (*8*) studied the excretion and storage sites of ^{131}I-PVP preparation A_1 using whole-body counting and body scanning and determining the rate of disappearance of polymer from the plasma; body scanning showed localization of radioactivity to the liver with half the injected material remaining in the body. Tothill discussed the irradiation effects of prolonged retention of ^{131}I-PVP within the liver assuming that all the polymer retained in the body was selectively stored in this organ and that the tissue radiation dose was 0.05 rad/μc ^{131}I. This assumption is contrary to the findings reported here and the observations of previous workers (*12,13*). The serious difficulty in calculating organ radiation dose is the progressive increase in PVP localization of the highest-molecular-weight material to the liver and spleen (Table 4), probably taking weeks to reach completion (*12*).

It seems reasonable to assume from the data that there are two phases of PVP clearance: the first involves an even distribution throughout the body with a half-life of 12 hr; the second occurs when a maximum of 5% of the injected material is concentrated in the liver with a biological half-life identical to the physical half-life of the isotope. For a 50-kg patient whose liver weighs 1.5 kg, the calculated radiation dose to this organ is 0.0046 rad/μc of ^{131}I-PVP administered. A change of isotope from ^{131}I to ^{125}I would reduce the radiation to 0.0013 rad/μc, and a test dose of 20 μc would deliver less than 0.1 rad to the liver. Therefore for each isotope a much lower radiation dose is received in the liver than from radioiodinated human serum albumin (*14*) and the dose is well within accepted levels.

SUMMARY

The storage sites of radioactively labeled polyvinylpyrrolidone (PVP) with different molecular weights have been studied in different species. PVP with a molecular weight of less than 45,000 is rapidly excreted by the kidney while higher-molecular-weight material is selectively stored in the reticuloendothelial system; 4–6% of the administered dose is retained in the liver. The irradiation of this organ after the administration of 20 μc of ^{125}I or ^{131}I PVP does not constitute an irradiation hazard.

ACKNOWLEDGMENT

During this work, B. Hulme received a grant from the United Birmingham Hospitals Endowment Fund. We wish to thank Mr. Willans of the Radiochemical Centre, Amersham, for supplying the radioactively labeled PVP.

REFERENCES

1. HECHT, G. AND WEESE, H.: Periston, ein neuer Blutflüssigkeitsersatz. *Munchen Med. Wochschr.* **90**:11, 1943.

2. KLEIDERER, I. C.: Blood substitute technical summary report (P.B. report No. 67620), U.S. Dept. of Commerce, 1947.

3. SCHOEN, H.: Organveränderungen beim Saügling nach Zufuhr von Periston. *Klin. Wochschr.* **27**:463, 1949.

4. AMMON, R. AND MULLER, W.: Der Einfluss hoher Peristongaben auf den Kaninchenorganismus unter besonderer Berücksichtigung der Speicherorgane. *Deut. Med. Wochschr.* **74**:465, 1949.

5. GORDON, R.: Exudative enteropathy: abnormal permeability of the gastrointestinal tract demonstrable with labelled polyvinylpyrrolidone. *Lancet* **I**:325, 1959.

6. TAUXE, W. N., SEDLACK, R. E., PITLYK, P. J. AND KERR, F. W. L.: Preliminary report on the localization of brain lesions with ^{131}I labeled polyvinylpyrrolidone. *Proc. Staff Meetings Mayo Clinic.* **37**:109, 1962.

7. HULME, B. AND HARDWICKE, J.: The measurement of renal permeability using labeled macromolecules. *Proc. Roy. Soc. Med.* **59**:509, 1966.

8. TOTHILL, P.: The retention by the body of ^{131}I polyvinylpyrrolidone and its effect on radiation dose. *J. Nucl. Med.* **6**:582, 1965.

9. LAURENT, T. C. AND KILLANDER, J.: A theory of gel filtration and its experimental verification. *J. Chromatog.* **14**:317, 1964.

10. WALLENIUS, G.: Renal clearance of Dextran as a measure of glomerular permeability. *Acta Soc. Med. Upsalien.* Supplement 4, 1954.

11. ROOTVELT, K.: Direct intravenous injection of ^{51}Chromic chloride compared with ^{125}I polyvinylpyrrolidone and ^{131}I albumin in the detection of gastrointestinal protein loss. *Scan. J. Clin. Lab. Invest.* **18**:405, 1966.

12. RAVIN, H. A., SELIGMAN, A. M. AND FINE, J.: Polyvinylpyrrolidone as a plasma substitute. Studies on its excretion, distribution and metabolism. *New Engl. J. Med.* **247**:921, 1952.

13. MEIJER, A. E. F. H.: The enlargement of the liver and spleen in mice of the 0_{20} (Amsterdam) strain caused by the storage of macromolecular substances — 1. The storage of the macromolecular substances dextran and polyvinylpyrrolidone in the liver and spleen. *Biochem. Pharmacol.* **11**:1,137, 1962.

14. DYKES, P. W.: Studies on the rates of distribution and catabolism of human serum albumin and on the pathogenesis of ascites. M.D. Thesis. University of Birmingham, 1964.