THE ELIMINATION OF ALBUMIN, POLYVINYLPYRROLLIDONE AND DEXTRAN FROM THE CIRCULATION IN SHEEP

N.C. OWEN*, A. IMMELMAN** AND DRICKY GRIB **

SUMMARY

The rates of elimination of iodinated human serum albumin (ALBUMIN-^{125}I), polyvinylpyrrolidone (PVP-^{125}I); and tritiated dextran (DEXTRAN - METHOXY-^{3}H) (mol masses of 69 000 - 72 000, 30 000 - 40 000 and 60 000 - 90 000 respectively) from the circulation of sheep were studied; albumin and PVP initially disappeared from the circulation rapidly having half-life times ($t_{\frac{1}{2}}$) of 10,5 ± 3,7 and 43 ±45 hours respectively. This phase is regarded as being due to equilibration within the initial volume of distribution, the rate being determined primarily by the relative mol. mass of the molecule. Hereafter PVP-^{125}I was eliminated considerably faster ($t_{\frac{1}{2}}$ = 176 ±39 h) principally via the kidneys. The limifed data available for dextran-^{3} H suggests that this particular substance is rapidly excreted via the kidneys ($t_{\frac{1}{2}}$ = 9 h)

INTRODUCTION

The elimination of various large molecular mass substances from the circulation of different animals has been studied and has led to their use as plasma expanders in clinical procedures[1,2]

Thus Giebish, Lauson and Pitts studied the renal excretion of various dextrans by dogs[4] while Hecht and Scholtan[5] followed the excretion of polyvinylpyrrolidone via the kidney of man, dog and rabbit. These substances expand the plasma volume by being retained in the circulation for a period of time[6]. Consequently their elimination from the blood of sheep was compared to that of albumin with a view to their use as markers in capillary permeability studies.

MATERIALS AND METHODS

Experimental animals and labelled substances

Twelve adult Merino grade whethers were used in these studies. The elimination of albumin was followed in six sheep, that of polyvinylpyrrlolidone in four sheep and dextran in two sheep.

The isotopically labelled substances used and their average molecular masses are:

Iodinated polyvinylpyrrolidone (PVP-^{125}I); mol. mass 30 000 - 40 000 (Amersham);

Iodinated human albumin(Alb^{125}I); mol. mass 69 000 - 72 000 (Amersham);

Tritiated dextran (Dextran methoxy-^{3}H); mol. mass 60 - 000 - 90 000 (New England Nuclear).

Experimental procedure

The sheep were housed individually in metabolism crates designed for the automatic separation of urine and faeces.

At the commencement of each experiment 100μ Ci of isotope were injected into the jugular vein, followed by the collection of venous blood samples 7 ml in volume using heparin as anticoagulant at selected intervals of increasing duration (Fig. 1). Total urine excretion was collected at 12 hourly intervals; the volume noted and a sample taken for analysis. Where iodinated isotope was to be administered, thyroid uptake of labelled iodine was blocked by injecting 10 ml of a 10% w/v Na I_2 solution intravenously, 24 hours in advance.

Radioactivity in the case of Alb-^{125}I and PVP-^{125}I was determined by counting whole blood or urine in a Philips Automatic Welltype Scintillation Detector (Type PW 4003) using a 4,5 x 5,0 cm NaI/Te^{4} crystal. All samples were counted on the same day to obviate correction for decay.

Free-^{125}I was estimated in the urine of a sheep injected with PVP-^{125}I by passing a quantity of urine through an Amicon Diaflo ultrafiltration membrane with limiting pore size equivalent to 30 000 mol. mass. The ultrafiltrate was then counted as before.

Dextran-^{3}H activity was determined by pipetting 0,5 ml. of plasma or 0,1 ml of urine into a 10ml of a commercial scintillation cocktail (Scintisol) and counting in a Packard Tri-carb scintillation spectrometer with Activity Analyser to correct for quenching.

RESULTS

The relative rates of excretion of the three labelled substances via the kidneys are illustrated by the data given in table I. The blood activity is the mean of the 30 minutes, 8 hour and 24 hour samples taken as corresponding to the period of urine collection. As the figures have little absolute value, a comparative figure is given as the ratio blood counts: urine counts for the three markers.

TABLE 1: RELATIVE EXCRETION RATES OF LABELLED COMPOUNDS OVER A 24 HOUR PERIOD

Isotope	Activity (c p m) ml		Ratio
	Blood	Urine	Blood:Urine
Albumin^{125}I	27,9 x 10^3	0,97 x 10^3	29,4
PVP^{125}I	28,4 x 10^3	3,8 x 10^3	9,3
Dextran-^{3}H	21,9 x 10^3	583,2 x 10^3	0,38

* Dept. Physiology, Fac. Dentistry, Univ. West. Cape, Bellville.

** Dept. Physiological Sciences, Faculty of Veterinary Science, University of Pretoria, Box 12580, P.O. Onderstepoort, 0110.

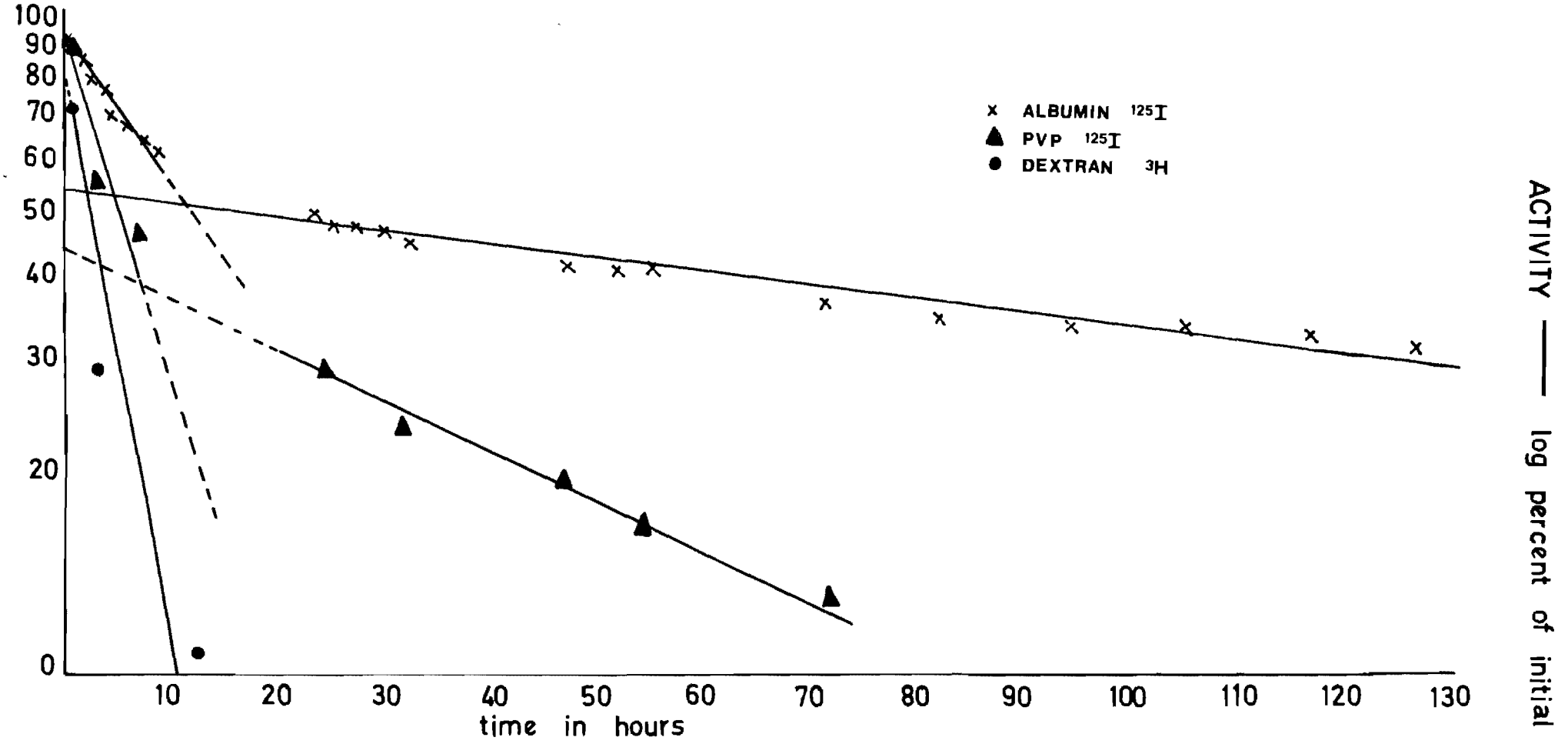

Fig. I DISAPPEARANCE OF ALBUMIN ^{125}I PVP ^{125}I AND DEXTRAN ^{3}H FROM BLOOD OF SHEEF

The ratios show marked differences between the substances regarding the rate of renal excretion over the first 24 hours. Surprisingly, Dextran-^{3}H is excreted very rapidly into the urine when compared to either PVP or albumin. PVP-^{125}I was nevertheless excreted considerably faster than albumin.

The rates of elimination from the blood were estimated by determining the half-life times ($t\frac{1}{2}$) for the different substances. The logarithm of the blood activity was plotted against the time of sampling. Examination of the data suggested a two component disappearance curve, each of which appeared to be almost linear. The first component represented a phase of rapid disappearance which gave the initial blood concentration when extrapolated back to zero time. All subsequent counts were then expressed as a percentage of the initial concentration. Fig. I shows the mean logarithm of the blood activity for all sheep versus the time of sampling.

The results show that Dextran is rapidly eliminated from the blood, PVP less so, while albumin persists at relatively high levels over an extended period of time.

The half-life times ($t\frac{1}{2}$) for the elimination of each of the substances have been estimated from the data obtained from the individual sheep and are shown as means ±1 SD where applicable (Table II)

TABLE II: HALF-LIFE TIMES FOR THE ELIMINATION OF LABELLED COMPOUNDS FROM THE BLOOD OF SHEEP

Isotopes	Half-life times in hours **	
	First component	Second component
Albumin-^{125}I	10,5 ± 3,7 (n=6)	175,7 ± 3,9 (n=6)
PVP^{125}I	4,3 ± 1,5 (n=4)	25,5 ± 3,6 (n=4)
Dextran-^{3}H	—	9*

* Individual values : 8,75 and 9,25 h. n = number experiments
** Values given as means ± 1 standard deviation.

Free iodine was estimated by ultrafiltration in the urine of a sheep injected with PVP-^{125}I and found to be approximately 1,9%. This value was within the specifications of the manufacturer, thus allowing the conclusion that degradation of the PVP had not occurred.

DISCUSSION

The elimination rates of the three markers (Albumin-^{125} I, PVP-^{125}I and Dextran-^{3}H) appeared to be exponential, giving almost linear relationships on a semi-logarithmic plot. Between sheep variation for albumin and PVP elimination was relatively small as evidenced by the biological half-time standard deviations. These two substances disappeared from the circulation in a manner suggesting two distinct processes (a) an initial rapid phase presumably representing equilibration in the final distribution volume; and (b) gradual removal by other means.

Dextran and PVP appear to be eliminated chiefly via the kidneys while albumin is persumably degraded and the free iodine excreted into the urine.

The findings with regard to the slowly excreted component of PVP-^{125}I are essentially in agreement with those of Hecht and Scholtan[5] for dog, man and rabbit. They estimate some 80% of the administered PVP was excreted into the urine during the first 3 days. Our data supports the direct passage of PVP-^{125}I into the urine, giving a biological half-life time of 25 ±3,6 h in sheep.

The nature of the disappearance of labelled albumin from the circulation is very similar to that reported for man[1] and rabbit[3]. However, the half-times required for equilibration in the extracellular fluid of man were three and 24 h using homologous albumin[1] versus an equilibration time of 40 to 60 h for rabbits[3]. Assuming the initial rapid declining phase of activity to represent equilibration in our studies, a biological half-life time of 10,5 ±3,7 h was obtained for heterologous albumin in sheep. The corresponding value for PVP was 4,3 ±1,5 h. The difference in equilibration time may reflect differing final volumes of distribution or the effect of the molecular mass differences between these two substances. The smaller molecule (PVP) passing more rapidly through the capillary walls. Similar differences were observed for albumins and globulins in rabbits[3].

The biological half-life of 176 ±39 h for human serum albumin in sheep corresponds closely to that of 160-230 h for homologous albumin in rabbits[3] and would appear to be a suitable marker for volume distribution and permeability studies. However, antigenicity would preclude repeated use in the same animal. Presumably urinary activity represents free^{125}I detached from the albumins[1,3].

A striking feature of these results is the relatively rapid urinary excretion of dextran with a molecular mass of 60 000-90 000. This contrasts with dextran clearance studies in dogs which showed a rapidly diminishing rate of excretion at mol. masses exceeding 30 000[4]. In view of the use of dextrans as plasma expanders generally[6] this aspect required closer study in the sheep.

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