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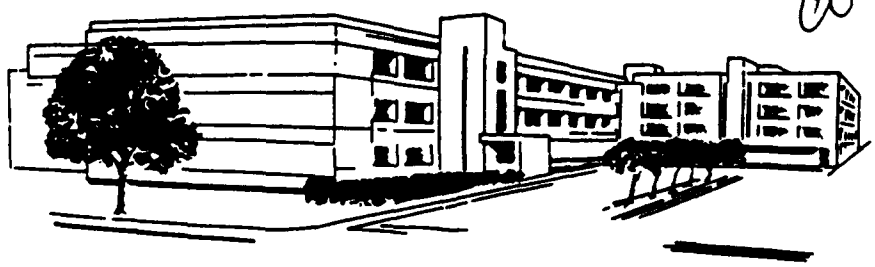
INSTITUTE REPORT NO. 427

Plasma Clearance and Tissue Distribution of Dextran Following Infusion of 7.5% NaC1/6% Dextran-70 Euvolemic and Hemorrhaged Rabbits

Michael A. Dubick,
Barbara A. Ryan,
James J. Summary,
and Charles E. Wade

Division of Military Trauma Research

November 1989



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Plasma Clearance and Tissue Distribution of Dextran Following Infusion of 7.5% NaC1/6% Dextran-70 to Euvolemic and Hemorrhaged Rabbits (Institute Report 427)--M. Dubick, et al

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Donald G. Corby 28 Nov 89

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UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release, Distribution is Unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): Institute Report No. 427	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Division of Military Trauma; 6b. OFFICE SYMBOL (If applicable): SGRD-ULT-M	7a. NAME OF MONITORING ORGANIZATION: U.S. Army Medical Research and Development Command
6c. ADDRESS (City, State, and ZIP Code): Letterman Army Institute of Research, Presidio of San Francisco, CA 94129-6800	7b. ADDRESS (City, State, and ZIP Code): Ft. Detrick, Frederick, MD 21701-5012
8a. NAME OF FUNDING/SPONSORING ORGANIZATION; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 63807A; PROJECT NO. D836; TASK NO. AX; WORK UNIT ACCESSION NO. Ø87

11. TITLE (Include Security Classification)

(U) Plasma Clearance & Tissue Distribution of Dextran following infusion of 7.5% NaCl/6% Dextran-70 to euvolemic and hemorrhaged rabbits (U)

12. PERSONAL AUTHOR(S)

M Dubick, B Ryan, J Summary, and C. Wade.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Institute	FROM _____ TO _____	October 1989	22

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	(U) Dextran-70, hypertonic resuscitation, hemorrhage, rabbits, liver, lung, kidney (U)

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Dextran metabolism was evaluated in euvolemic and hemorrhaged rabbits following administration of a 7.5% NaCl/6% Dextran-70 (HSD) solution. Control rabbits and those bled 8 ml/kg body weight were infused i.v. with 4 ml/kg of HSD or HSD containing ~~1 μCi/ml of~~ ^{14}C-Dextran-70. Blood samples were withdrawn prior to and 0.17, 0.5, 1, 2, 4, 6, 24, 48, 72 and 96 hours after HSD infusion. Although peak serum dextran concentrations were about 29% higher in hemorrhaged rabbits than in controls, in both groups of rabbits dextran was cleared from serum with a half-life of about 7.4 h. In addition, dextran was distributed throughout the blood volume and did not bind to serum proteins. At the end of the 96 h experimental period, concentrations of radiolabeled dextran were 20-fold higher in liver from both groups of rabbits, in comparison to spleen, lung and kidney. The ^{14}C-dextran in liver was associated with the cytosolic fraction and was not associated

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
XXX UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: COL Donald G. Corby, MC, Commanding	22b TELEPHONE (Include Area Code): 561-3600; 22c. OFFICE SYMBOL: SGRD-ULZ

DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

Abstract (Cont)
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ABSTRACT

Dextran metabolism was evaluated in euvolemic and hemorrhaged rabbits following administration of a 7.5% NaCl/6% Dextran-70 (HSD) solution. Control rabbits and those bled 8 ml/kg body weight were infused i.v. with 4 ml/kg of HSD or HSD containing 1 μCi/ml of ^{14}C-Dextran-70. Blood samples were withdrawn prior to and 0.17, 0.5, 1, 2, 4, 6, 24, 48, 72 and 96 hours after HSD infusion. Although peak serum dextran concentrations were about 29% higher in hemorrhaged rabbits than in controls, in both groups of rabbits dextran was cleared from serum with a half-life of about 7.4 h. In addition, dextran was distributed throughout the blood volume and did not bind to serum proteins. At the end of the 96 h experimental period, concentrations of radiolabeled dextran were 20-fold higher in liver from both groups of rabbits, in comparison to spleen, lung and kidney. The ^{14}C-dextran in liver was associated with the cytosolic fraction and was not associated with any membrane structures. Molecular weight distribution of the serum dextran revealed no significant metabolism over the 6 h post-infusion period assayed. After 96 h, radiolabeled dextran in liver showed some degree of metabolism and dextranase activity in liver was markedly higher than in the other tissues assayed. Other than in the lung, no significant differences in dextranase activity were observed between the two groups of rabbits. These studies indicate that dextran infused as HSD does not associate with any protein fractions, is found only in low concentrations in tissue, and has a serum half-life adequate to serve as a useful plasma volume expander.

Plasma clearance and tissue distribution of dextran following infusion of 7.5% NaCl/6% Dextran-70 to euvolemic and hemorrhaged rabbits -- Dubick et al.

INTRODUCTION

Since the introduction of dextran solutions as plasma volume expanders over 40 years ago, a number of studies have been concerned with its metabolic fate. Histological evaluations in laboratory animals and Korean battle casualties observed the primary tissue distribution sites of dextran to be the kidneys, liver, leukocytes and spleen (1-3). Initial reports into the clearance of intravenously infused dextran solutions observed that in normal children and adults, plasma disappearance and urinary excretion of dextrans were inversely proportional to their average molecular weight (4-8). Subsequent investigation found that dextrans were primarily excreted through the kidney (4-6). Glomerular filtration of dextrans with molecular weights >50-60,000 was very low and dextran was neither reabsorbed nor secreted by the renal tubules (4-6,9).

Previous studies with Dextran-60 in newborns and young children observed a plasma clearance that appeared biphasic in nature, with a half-life ($t_{1/2}$) of 3 h in the initial phase, and 52 h in the second phase (10). In contrast, Arturson, et al. (4) reported a serum $t_{1/2}$ of 10.7 h infants and 6.2 h in children. In addition, 31-47% of a dose of Dextran-60 or 70 was excreted in urine in the first 24 h (7,11-13).

Despite a large body of literature, few studies have addressed dextran metabolism in the hypovolemic states in which it would be employed. In a controlled hemorrhage study in humans (bled 1 liter over 15 min), no significant differences in plasma dextran concentrations or urinary dextran excretion were observed between these individuals and their normovolemic counterparts at the times assayed (14). In a recent study with a new hypertonic saline (7.5%)/Dextran-70 (HSD), Holcroft, et al. (15) reported that approximately 30% of the dextran remained in plasma 24 h following infusion of HSD to trauma victims, but no controls were included in this study for comparison.

Dextran preparations have varied over the years with regard to the bacterial strain synthesizing the

dextran and the narrowing of its molecular weight range (16). Consequently, variable results have been reported in experimental animals regarding the tissue distribution and metabolism of dextran (17-21). In addition, since Gray (22) suggested that dextrans can be metabolized by mammals and the components utilized, a number of investigators have reported the presence of dextranases in mammalian tissue, including human (23-25).

With a lack of information concerning dextran metabolism in the hypovolemic state, and renewed interest in hypertonic resuscitation fluids containing Dextran-70, the present study investigates dextran clearance, tissue distribution and dextranase activity following administration of HSD to both euvolemic and hemorrhaged rabbits.

MATERIALS and METHODS

Animals and treatment

Adult, female New Zealand white rabbits (Elkhorn Rabbitry, Watsonville, CA) initially weighing 2.5 to 3.5 kg, were randomly assigned to either the hemorrhage (n=10) or control (n=8) group. Rabbits were catheterized via the middle ear artery and in the hemorrhaged group, bled 8 ml/kg body weight over a 15 min period to mimic a moderate hemorrhage. After a 30 min stabilization period, rabbits in both groups were infused intravenously with 4 ml/kg body weight with the HSD solution (Lot No.: NC 54845) (AB Pharmacia, Uppsala, Sweden) or the same volume of HSD containing 5 μCi/kg carboxyl-^{14}C-Dextran-70 (Lot No.: 2275-289, sp. act. 0.8 mCi/g); DuPont-New England Nuclear, Boston, MA). Blood samples were withdrawn prior to and 0.17, 0.5, 1, 2, 4, 6, 24, 48, 72 and 96 h after the HSD infusion. In experiments where no radioactive dextran was infused, a blood sample was also drawn 7 days after HSD infusion. After each blood sample, an equivalent volume of saline was infused back into the animal to help maintain plasma volume. During the experimental period, rabbits were individually housed in metabolic cages. Serum samples were frozen at -20°C until assayed.

Dextran Measurements

Total carbohydrate concentrations in serum and urine were determined by the anthrone reaction (26) following precipitation of serum with 10%

tricholoacetic acid (TCA) and oxidation of endogenous glucose with glucose oxidase (27). The resultant product represented dextran concentrations. Final concentrations were corrected for hemodilution induced by HSD during the first 6 h after infusion by multiplying the concentration by the ratio of the hemoglobin concentration prior to and at the specific time after infusion. Previous studies using Evan's Blue Dye to estimate plasma volume (28) confirmed that in both euvolemic and hemorrhaged rabbits, plasma volume returned to pre-HSD infusion levels by 24 h after its administration (data not shown). In addition, in the experiments employing ^{14}C-dextran, an aliquot of serum and deproteinized serum was counted for radioactivity by liquid scintillation. Data were expressed as dpm/ml serum or dpm/mg dextran.

Gel Filtration

Serum samples were deproteinized with TCA, neutralized and the protein-free aliquots applied to a 0.9 x 87 cm column of Sephadex 200/100 equilibrated with 0.3% NaCl and eluted with the same solution (29). Fractions were collected and assayed for anthrone-reactive substances as described above or counted for radioactivity by liquid scintillation. These studies quantitated the molecular weight distribution of the dextran fractions to detect metabolism of HSD following its infusion.

Tissue Distribution

The tissue distribution of ^{14}C-dextran in liver, lung, kidney and spleen was determined at the end of the 96 h experimental period. These tissues have been shown previously as primarily involved in dextran metabolism (16-18). Tissues were oxidized in a Packard Tricarb Oxidizer (Packard Instruments, Downner's Grove, IL) and data expressed as dpm/g tissue. In other experiments, the uptake and binding of ^{14}C-Dextran-70 to crude membrane fractions were determined in vitro with liver. Crude liver membrane fractions were prepared according to the method of Dangott, et al. (30). Membranes were then incubated with the radiolabeled dextran in the absence or presence of a 100 fold excess of cold Dextran-70, for 0, 5, 10, 15 and 30 min. After centrifuging in a microfuge, the resultant pellet was washed 4 times with saline. The final pellet was resuspended in the membrane buffer and an aliquot counted for radioactivity by liquid scintillation. In other studies, liver slices in organ culture were incubated with the radiolabeled dextran

for 0, 15, 30, 60 and 90 min. Liver was then homogenized in 5 volumes of 0.2 M Tris-HCl buffer, pH 7.4 and an aliquot of the whole homogenate, 10,000 x g supernatant (centrifuged 10,000 x g for 30 min) and the resultant pellet was counted for radioactivity. Protein was determined by the method of Bradford (31) and data expressed as dpm/mg protein to evaluate dextran uptake by liver.

Dextranase Activity

To further evaluate dextran metabolism, dextranase (E.C.3.2.1.11) activity in liver, lung, kidney and spleen was determined at the end of the 96 h experimental period according to the method of Janson and Porath, as described in the Worthington Manual, 1988 (Worthington, Freehold, NJ). Activity was expressed as mU/mg protein.

Statistical Analysis

The radioisotope dilution technique was employed to evaluate pharmacokinetic parameters of HSD metabolism in both the euvolemic and hemorrhaged rabbits. The best-fit for ^{14}C-Dextran-70 disappearance curves were plotted on a semilog scale and analyzed by least squares non-linear regression (32) to determine half-life and apparent volume of distribution. A BMDP non-linear regression program was employed for kinetic analysis (33). The best fit of the data was described by a 1-compartment model defined by the equation: $Y=Ae^{-kt}$, where "A" is the amount of drug administered at time 0, "k" is the rate constant of elimination, and "t" is time. Statistical comparison of the kinetic parameters derived, dextranase activity, and tissue distribution between the two groups was by Student's t-test (32). Analysis of variance was used to analyze liver membrane binding and liver uptake of dextran with time as the independent variable (32).

RESULTS

Dextran Concentrations and Clearance

In the present study, rabbits were weight matched so that similar amounts of dextran as HSD were administered to both the euvolemic control and hemorrhaged groups. Nevertheless, maximum serum dextran concentrations were 29% higher in hemorrhaged rabbits than in controls (Table I). In both groups of rabbits, serum dextran concentrations were generally undetectable after 48 to 72 h.

In both the euvolemic and hemorrhaged rabbits, dextran concentrations in serum were highest at the initial sampling time and disappeared from serum at a rate corresponding to a half-life of 7.4 h (Fig. 1, Table I). Clearance rates of the radiolabeled Dextran-70 paralleled the disappearance of dextran in HSD (data not shown). As shown in Fig. 2, ^{14}C-Dextran-70 contained lower molecular weight components than the Dextran-70 in HSD. Therefore, only the HSD data are presented. Further evaluation indicated that dextran was not associated with serum proteins. In addition, the apparent volume of distribution (Vd) calculated suggested that dextran was distributed throughout the blood volume, and the observed differences in Vd between the hemorrhaged and control rabbits reflected differences in blood volume due to hemorrhage (Table I).

Gel filtration chromatography was employed to detect changes in the molecular weight distribution of the administered dextran for up to 6 h after infusion. At each time point assayed, no differences in molecular weight distribution were observed in serum between hemorrhaged or euvolemic rabbits. In comparison to the native ^{14}C-Dextran-70 infused, the molecular weight profile of ^{14}C-dextran at 6 h post infusion, showed a decrease in low molecular components (Fig. 2).

Tissue Distribution

At the end of the 96 h experimental period, concentrations of ^{14}C-Dextran-70 were determined in liver, kidney, spleen and lung from both groups of rabbits. As shown in Fig. 3, concentrations of labeled dextran, expressed as dpm/g tissue, were similar in lung, spleen and kidney, whereas they were about 20-fold higher in liver. In liver these concentrations of dextran were approximately 39 μg/g or < 1% of the infused dose. Again no significant differences were observed between the 2 groups of rabbits (Fig. 3).

Since ^{14}C-dextran concentrations were markedly higher in liver, other experiments examined the binding of dextran to liver as well as its uptake. ^{14}C-Dextran-70 bound rapidly to crude liver membrane preparations, but the degree of binding at each time point was not higher than the background bound-to-free ratio (B/F) (Table II). In addition, the binding could not be displaced by over 100-fold excess cold Dextran-70, further suggesting that the binding was non-specific in nature. Studies of ^{14}C-Dextran-70 uptake

into liver observed that the highest radioactivity was found in the cytosolic fraction and was not associated with protein or membrane structures (Table II). In addition, in vitro uptake did not increase significantly over time. Evaluation of the molecular weight distribution of ^{14}C-Dextran-70 in liver 96 h after Dextran-70 infusion indicated that the peak molecular weight was around 40,000, suggesting some degree of metabolism.

Dextranase Activity

Additional experiments determined the dextranase activity in liver and the other tissues assayed. The highest dextranase specific activity, expressed as mU/mg protein, was found in liver and kidney (Fig. 4). Of the tissues assayed, the lowest activity was found in lung. Dextranase activity in liver and spleen from hemorrhaged rabbits was about 25% higher than in tissue from control rabbits, but the differences were not statistically significant (Fig. 4). In contrast, lung dextranase activity was 19% lower in hemorrhaged rabbits than in controls (Fig. 4).

DISCUSSION

Recent years have seen renewed interest in the use of hypertonic solutions to treat hemorrhagic shock. In dogs (34) and sheep (35), hypertonic (7.5%) NaCl improved cardiovascular function following hemorrhage, presumably by inducing a shift of intracellular fluid to the vascular compartment (36), although neural mechanisms may also be involved (37). This hemodynamic effect of 7.5% saline was only transient, and Smith, et al. (38) demonstrated that this effect could be sustained longer by adding dextran to the hypertonic saline solution. Subsequent studies in experimental animals have shown that small volume infusion of a hypertonic saline/dextran (HSD) solution was effective in restoring cardiovascular and renal function, and tissue blood flow, thereby improving survival following potentially lethal hemorrhage (36,39,40). In human field trials, HSD has also been reported to improve survival of trauma victims (24). Currently, HSD is being evaluated in clinical trials at 4 ml/kg body weight; a lower dose than that employed for either Dextran 40 or 70 in physiological saline (13,41).

Few studies have examined dextran metabolism following administration of HSD, and particularly in

the hypovolemic state. In the present study, dextran concentrations in serum peaked early and were significantly higher in the hemorrhaged rabbits in comparison to the euvolemic control. These results are consistent with our previous observations following administration of HSD at a dose of 4 ml/kg to hemorrhaged and euvolemic swine (40), and probably reflect differences in blood volume due to the hemorrhage. In addition, we observed the typical shift to higher molecular weight dextran components in serum over a 6 h period, consistent with previous reports that low molecular weight components are rapidly excreted by the kidney (7). This observation also suggests that the circulating dextran is not altered during this time period and agrees with reports that dextranases do not exist in serum (24). Thus, it appears that, at least in the 6 h period monitored, serum anthrone-reactive material represents the native Dextran-70 infused, and not metabolites of Dextran-70.

Despite the difference in serum concentrations, dextran clearance rates from serum were not significantly different between control and resuscitated hemorrhaged rabbits. The best fit of a graph depicting the change in serum dextran concentrations over time was described by a 1-compartment model and is consistent with the observation that dextrans distribute rapidly following i.v. administration (16,21). These data indicated that the serum $t_{1/2}$ of Dextran-70, administered as HSD, was 7.4 h and is consistent with previous reports in young children (4); but lower that the >12 h reported in normal adults following infusion of dextrans with molecular weights of 55,000 to 69,000 (7). It should be noted that this dextran preparation differs from clinical Dextran-70 and may account, at least in part, for the differences in serum $t_{1/2}$ observed.

As previously mentioned, studies with other clinical dextrans indicate that dextrans are primarily cleared through the kidney (5,6) which reflects the major route of metabolism. Thus, if renal function is not impaired by an induced hypovolemic state or corrected following resuscitation (40), it seems reasonable to assume that dextran clearance would be similar in both groups of rabbits.

It should be mentioned that some authors have reported that dextran clearance follows a biphasic pattern (10). In these situations it appears that the

first phase represents renal clearance, while the second phase presumably denotes dextran metabolism in tissues (10). It is reported that dextran metabolism is a slow process (16) and is insignificant with respect to the rate of renal clearance. Therefore, in the context of HSD as a resuscitation fluid for use in the field prior to transport to the hospital, this second phase of dextran clearance can be ignored.

The results from the present study indicated that after the 96 h experimental period, concentrations of ^{14}C-dextran were significantly higher in liver than in kidney, spleen, or lung. Previous studies in experimental animals reported that dextrans accumulated in liver, kidney and spleen (17-19), and that dextran concentrations in liver declined rapidly when plasma concentrations fell to undetectable levels (17). Swedin and Aberg (20), however, found that 18 h after an i.v. injection of Macrodex (6% Dextran-70 in normal saline; Pharmacia AB, Uppsala, Sweden), dextran concentrations were high in spleen, but low in liver and femoral muscle. In rabbits infused with Macrodex, dextran accumulated in polymorphonuclear leukocytes (1) with maximum amounts observed 3-4 h after injection; it appeared that the reticuloendothelial system, including that of the liver and spleen, played a major role in dextran metabolism (1,18,19). In mice it was also shown that 6-25% of ^{14}C-dextran of unspecified average molecular weight was expired in air as CO_2, 24 h after an i.v. injection (21). In addition, following an i.v. infusion of ^{3}H-dextran, the greatest accumulation was found in liver, spleen, kidney, and the gastrointestinal tract after 30 min, while after 24 h, most of the labeled dextran was in liver (19). Thus, results from the present study are consistent with previous observations on dextran accumulation in tissues. Although some storage of dextran in tissues has been observed by us and others, it does not appear to be associated with any toxic effects (16,42).

Gray (22) reported that the half-life of the ^{14}C carbon of an unspecified sized dextran was 6.1 days in mice after an i.v. infusion, suggesting that dextran can be metabolized by mammals, and its components reutilized by incorporation into the body's carbon pool. In the present study, dextranase activity was detected in all tissues assayed, with the highest activity, expressed as mU/mg protein, in liver, followed by kidney, spleen and lung. Although dextranase activity in lung from hemorrhaged rabbits

was significantly lower than in euvolemic controls, overall, dextranase activity did not appear to be significantly affected by hemorrhage. In agreement with previous reports (43), tissue dextranase also did not appear to contribute significantly to the observed rate of dextran clearance from serum. Thus, the data from the present study indicate that Dextran-70 infusion as HSD does not bind to proteins nor significantly accumulate in tissues. In addition it can be metabolized by tissues and in the hypovolemic state has a serum $t_{1/2}$ suitable for its use as a pre-emergency room volume expander.

Acknowledgement

The authors thank Dr. Virginia Gildengoren for the statistical analyses, SGT Juergen Pfeiffer and Dan Brooks for excellent technical assistance and Mrs. Theresa Wilson for preparation of the manuscript.

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TABLE I

Effect of HSD Administration on Peak Dextran Concentrations and Half-Life in Serum and Apparent Volume of Distribution in Euvolemic and Hemorrhaged Rabbits[1]

	Euvolemic	Hemorrhaged
Dextran Concentration (mg/dl)	504±36(8)	639±25*(10)
Half-Life (hours)	7.36±0.49(8)	7.36±0.32(10)
Volume Distribution (ml)	140.9±8.3(8)	116.5±6.7*(10)

[1]Data expressed as mean ± SE (n).

*P<0.05 from euvolemic control

TABLE II

Binding of ^{14}C-Dextran-70 to Rabbit Liver Membranes[1]

	Time After Addition of ^{14}C-Dextran-70 (min)				
	0	5	10	15	30
Binding (n=4) (Bound/Free/ mg protein) X10^{-4}	3.71±0.59	2.85±0.75	3.60±0.70	1.75±0.32	6.02±1.34

Uptake of ^{14}C-Dextran-70 in Rabbit Liver[1]

	Time After Addition of ^{14}C-Dextran-70 (min)				
Fraction	0	15	30	60	90
Whole Homogenate (n=4) (dpm/mg prot)	124±33	134±44	93±10	245±15	233±41
10,000 xg supernatant (n=4) (dpm/mg prot)	120±27	129±24	108±14	281±33	267±64
pellet (n=4) (dpm/mg prot)	36±5	50±5	39±4	96±10	96±7

[1] Data expressed as mean ± SE of quadruplicate determinations in duplicate experiments.

Figure Legend

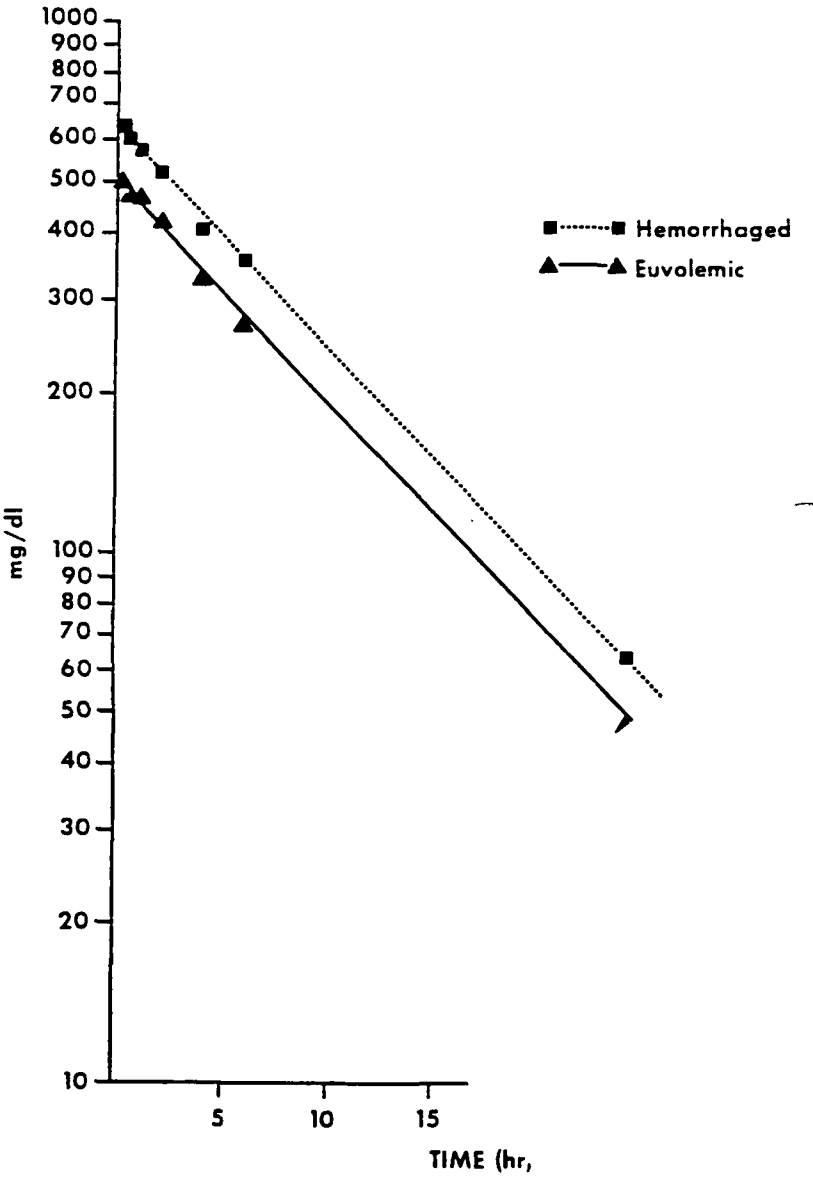

Figure 1: Dextran clearance from serum of euvolemic (solid line) and hemorrhaged rabbits (dashed line). Data represent mean ± SE of 8 euvolemic and 10 hemorrhaged rabbits. The SE are contained within the size of the symbols. Other details of the parameters that define the line are presented in the methods section.

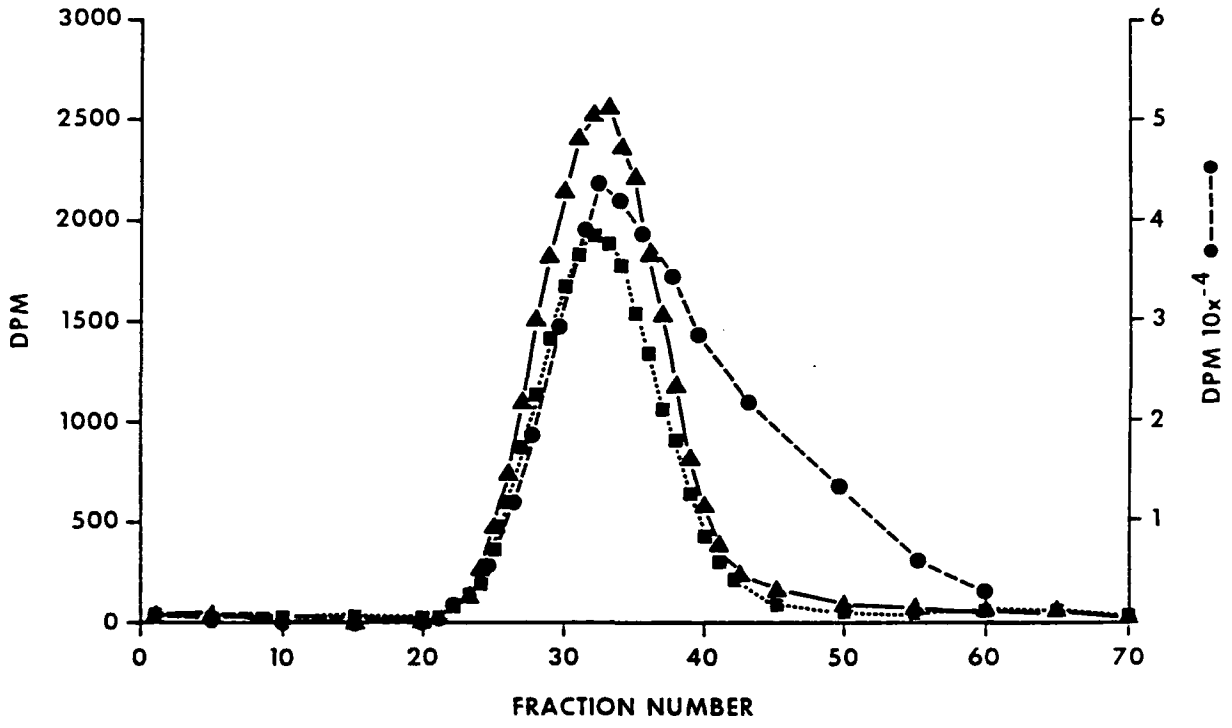

Figure 2: Molecular weight distribution of ^{14}C-Dextran-70 in serum from euvolemic and hemorrhaged rabbits. Profiles at 6h post infusion are compared with initial profiles of the native ^{14}C-Dextran-70.

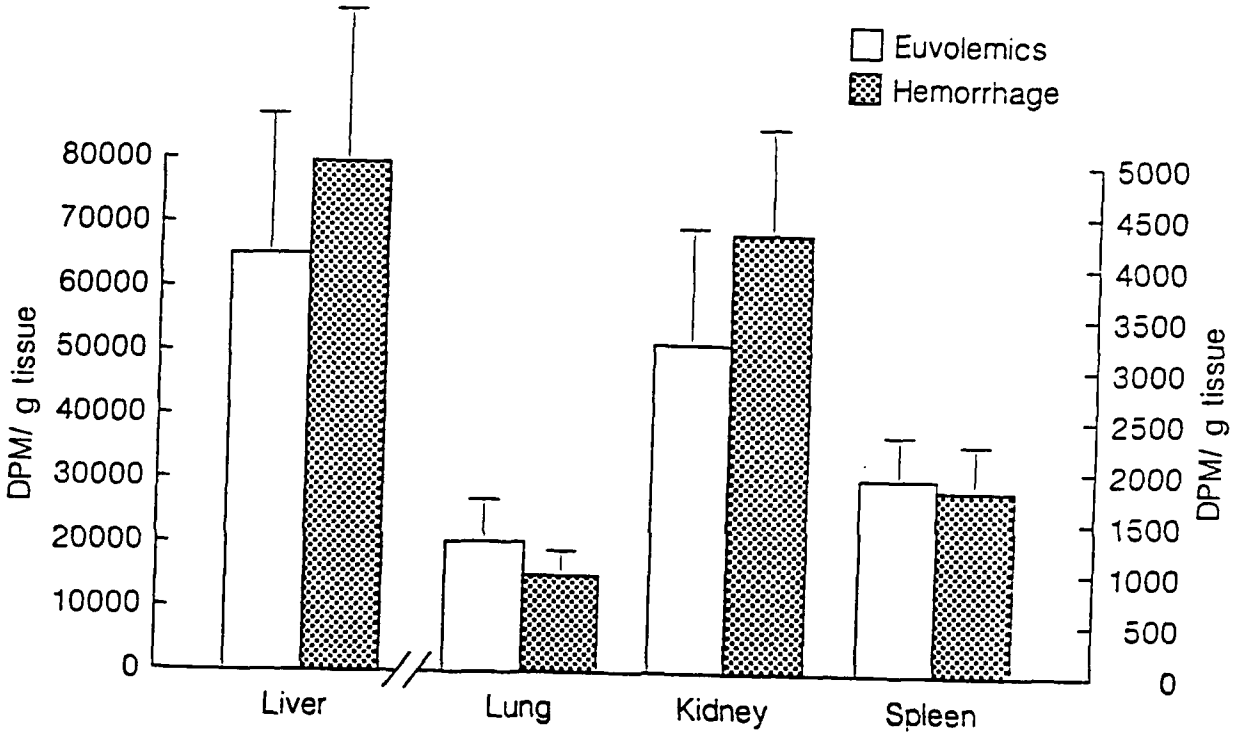

Figure 3: Tissue distribution of ^{14}C-dextran in euvolemic (n=5) and hemorrhaged (n=7) rabbits after 96h experimental period. Data expressed as mean ±SE of DPM/g tissue. Euvolemic, open bar; hemorrhaged, hatched bar.

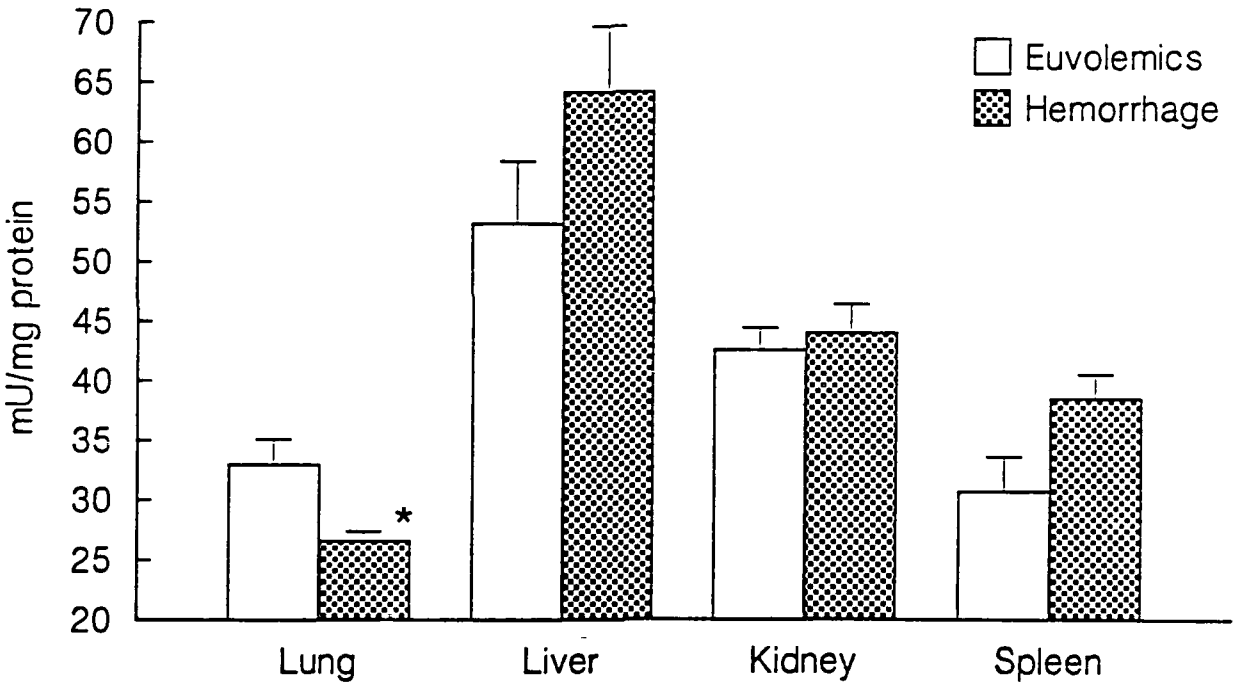

Figure 4: Tissue dextranase activity in euvolemic (n=7) and hemorrhaged (n=8) rabbits after 96h experimental period. Data expressed as mean ± SE of mU/mg protein. Euvolemic, open bar; hemorrhaged, hatched bar.

* P<0.05 from euvolemic control

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