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DISTRIBUTION AND RENAL EXCRETION OF DESFERRIOXAMINE AND FERRIOXAMINE IN THE DOG AND IN THE RAT*

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Abstract—In nephrectomized dogs given a single large intravenous dose of desferrioxamine the plasma concentrations declined rapidly. The decline could be described by the regression lines of three first-order reactions of which the first probably corresponds to equilibration and the second and third to metabolic degradation. The half-life of desferrioxamine in the nephrectomized dog was 76 ± 10 min. During the phase of rapid metabolic degradation metabolites of desferrioxamine accumulated in the blood. The volume of distribution of desferrioxamine in nephrectomized dogs was found to be 62·9 ± 6·7 per cent of bodyweight. Ferrioxamine was not degraded to any appreciable extent in nephrectomized dogs: after a large dose the plasma concentration fell very slowly—probably as a consequence of biliary excretion. The volume of distribution of ferrioxamine was equal to the volume of the extracellular compartment. Renal excretion of desferrioxamine in five out of six anaesthetized dogs occurred by glomerular filtration with tubular reabsorption at very low blood levels. In unanaesthetized rats desferrioxamine was excreted by glomerular filtration and tubular secretion. Tubular secretion seemed to be independent of tubular PAH secretion. Ferrioxamine was reabsorbed at low plasma levels both in anaesthetized dogs and in unanaesthetized rats: The reabsorptive process had a low Tm.

FERRIOXAMINE B is an iron-containing metabolite isolated from the culture fluid of *Streptomyces pilosus* Ettlinger and other actinomycetes. Its iron-free derivative desferrioxamine B, now generally called desferrioxamine. can be obtained by chemical methods or by culturing the micro-organism in iron-deficient media.[1] Ferrioxamine belongs to the general group of sideramines, which are growth factors for certain micro-organisms.[2] The constitution of ferrioxamine B, desferrioxamine B, and some of their metabolites has been elucidated (Fig. 1).

Since desferrioxamine has a very high affinity for ferric ions and a much lower affinity for other bivalent and trivalent metallic cations, it is used in clinical therapy as a specific chelating agent for iron.[10] As such it is extensively used in the treatment of pathological iron deposition encountered in primary and secondary haemochromatosis, in transfusion haemosiderosis, and in sideroachrestic anaemia, as well as in the treatment of iron intoxication.

The present experiments were undertaken in order to elucidate the overall distribution, the rate of degradation, and the mode of renal excretion of desferrioxamine B and its iron chelate after parenteral administration.

* Preliminary communications: Peters, Keberle, Schmid and Brunner,[20] Peters,[21]

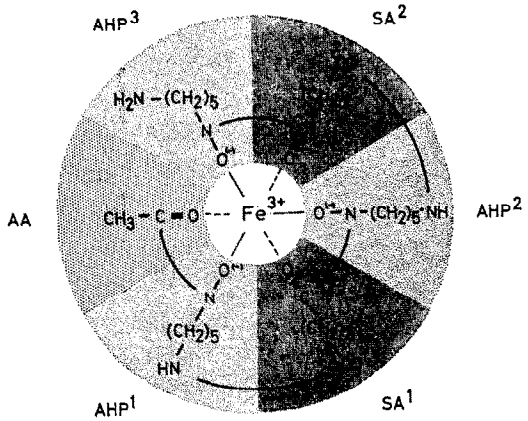

FIG. 1. Constitution of ferrioxamine B.[3-8] The whole molecule is composed of one molecule of acetic acid (AA), three molecules of 1-amino-5-hydroxylamino-pentane (AHP 1-3) and two molecules of succinic acid (SA 1-2). Desferrioxamine is an open-chain molecule with complete hydroxamic acid groups (-CO-N-) between AA-AHP[1], SA[1]-AHP[2] and SA[2]-AHP[2]. "Metabolite C"[9] of desferrioxamine
|
OH
results from oxidative deamination of AHP[3], which becomes -N-$(CH_2)_4$-COOH.

METHODS

Animal experiments

Experiments in dogs were carried out under pentobarbitone anaesthesia (35–40 mg/kg i.v., supplemented by additional small doses when necessary).

To measure the distribution volumes the dogs were nephrectomized from a mid-abdominal incision at the beginning of the experiment. Solutions were infused into a femoral vein, arterial blood samples were obtained from a femoral artery, and the blood pressure was recorded with a mercury manometer in a carotid artery. In experiments with desferrioxamine a dose of 150–300 mg/kg was infused intravenously as rapidly as possible. Rapid intravenous injection of desferrioxamine causes a fall in blood pressure in dogs which may be due to the liberation of histamine.[11] Usually only 10 per cent of the total dose could be infused in the first 30 min. In the course of the infusion vascular sensitivity decreases progressively: thus, 20–30 per cent of the total dose was tolerated in the second half hour and the remaining 60–70 per cent in the third half hour. Together with desferrioxamine a dose of inulin calculated to yield a plasma concentration of 20–50 mg/100 ml was infused. In two experiments, desferrioxamine randomly labelled with tritium was infused. The time at the end of the infusion was considered as t_0. Arterial blood samples were obtained at 2, 5, 10, 20, 30, 40, 50 and 60 min after t_0 and thereafter at hourly intervals. Blood samples were collected in heparinized tubes and were rapidly centrifuged. The plasma was analysed for desferrioxamine, total radioactivity, and inulin.

The logarithms of the blood concentrations of desferrioxamine and of total radioactivity were plotted as a function of time. The concentrations of inulin became stable one hour after t_0: thereafter, this time was considered as the equilibration period. The slope of the desferrioxamine concentrations in the blood against time showed a

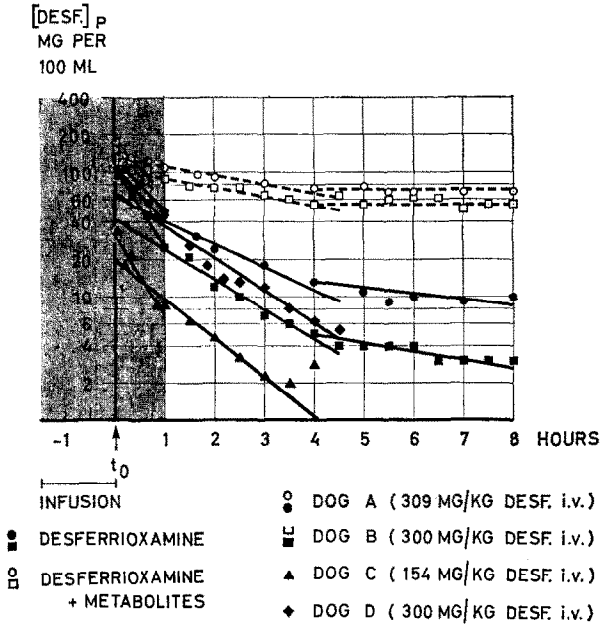

FIG. 2. Plasma concentrations of desferrioxamine B after the administration of a large i.v. dose to four nephrectomized dogs. In two experiments a small amount of desferrioxamine randomly labelled with ^3H was infused simultaneously with unlabelled material. The broken lines show concentrations of desferrioxamine and all its radioactive metabolites computed from total radioactivity and expressed as desferrioxamine base (mg/100 ml). The uninterrupted lines show concentrations of desferrioxamine determined by the chemical method and also expressed as mg base/100 ml of plasma. In dogs A-C the analytical method used measured desferrioxamine and "metabolite C", while in dog D desferrioxamine was determined.

definite break 3½ to 4 hr after t_0 (Fig. 2). Regression lines for log [desferrioxamine]/time were, therefore, calculated separately for the equilibration period, the period from 1 to 4 hr after t_0, and the period from 4 to 8 hr after t_0, according to the method of the least squares.[12] The biological half-life of desferrioxamine in the nephrectomized dog ($t/2$) was calculated from the slope of plasma concentrations against time between 1 and 4 hr after t_0 as:

$$t/2 = \frac{t.\ln 2}{\ln c_{t_0} - \ln c_t}$$

(c_t = concentration of desferrioxamine in the plasma at time t; c_{t_0} = hypothetical concentration at t_0 obtained by extrapolating the regression line between the first and the fourth hour back to t_0).

Experiments on the distribution of ferrioxamine were done in the same manner. The whole dose of 60–300 mg/kg of ferrioxamine could, however, be infused within 30 min, since the iron-containing compound is better tolerated.[11] Equilibration, as judged by the constancy of the inulin concentrations in the plasma, was achieved 90 min after t_0. The fall in the logarithms of the plasma concentrations of ferrioxamine after the equilibration period could be summarized by a single regression line (Fig. 2). $t/2$ was calculated from this line.

The renal clearances of desferrioxamine and ferrioxamine were measured in anaesthetized dogs in the same manner. Both ureters were cannulated for urine collection. Priming injections calculated to yield the desired plasma level were given as rapidly as tolerated, and were followed by intravenous infusions to maintain this level. After suitable clearance periods a second priming injection and infusion sufficient to maintain a higher plasma concentration were given. In some experiments this procedure was repeated several times. In all experiments the renal clearances of inulin and of *p*-aminohippurate (PAH) were measured simultaneously at plasma levels of inulin between 20 and 50 mg per cent.

The rate of urine flow was varied in some experiments by infusing a solution containing 10 per cent (w/v) mannitol and 0·9 per cent (w/v) NaCl. Urine collection periods varied from 10 to 40 min. with different rates of urine flow. Mid-period arterial blood samples were obtained for every period and analysed for desferrioxamine or ferrioxamine, inulin and PAH. In some experiments the renal extraction of desferrioxamine and inulin was also measured by analysing the renal venous blood samples obtained from a catheter introduced into the renal vein via the jugular vein and superior and inferior vena cava: the position of the catheter tip was checked at autopsy.

In rats the renal clearances of desferrioxamine or of ferrioxamine were measured simultaneously with the renal clearances of inulin and of PAH in unanaesthetized animals following the procedure of Cotlove[13] with modifications described previously.[14] Suitable blood concentrations of the sideramine or its iron-free analgoue were obtained by intravenous priming injections followed by intravenous infusions. The infusions were given in isotonic saline at a rate of 0·2 ml/rat min.

Urine collection periods lasted from 25 to 40 min. At every given plasma concentration of the drugs investigated two to three clearance periods were obtained for each rat. Since approximately 1·5 ml of plasma was needed for analysis, blood samples had to be obtained at the end of the last clearance period by exsanguinating the animals from the abdominal aorta under ether anaesthesia. An equilibration period of 60–75 min was allowed to elapse between the priming injection and the first clearance period. In order to ascertain whether the end-period plasma concentrations differed markedly from the mid-period values, one third of the animals comprising each group of 12 rats were exsanguinated at the end of the equilibration period. Of the remaining animals those whose final plasma concentration of desferrioxamine or ferrioxamine differed by more than 20 per cent from the mean pre-clearance value were exluded from the calculations. For the remaining animals the mid-period concentration was assumed to be the arithmetic mean of the preclearance mean and the individual end-period concentration. A similar procedure was used in those groups in which the renal clearances were measured at two different plasma concentrations.

Chemical methods

Inulin concentrations were estimated by the resorcinol-iron method.[15] PAH was measured by a modification of the Bratton-Marshall procedure.[16]

The analytical procedure for measuring concentrations of desferrioxamine or ferrioxamine in plasma and urine will be described in detail elsewhere.[17] Briefly, ferrioxamine was extracted quantitatively into benzyl alcohol from aqueous solutions saturated with NaCl and estimated spectrophotometrically at 430 mμ. Desferrioxamine

concentrations were measured by adding a slight excess of ferric chloride at an acid pH: the ferrioxamine was then extracted into benzyl alcohol and analysed photometrically. The difference in the extinction values between one sample after addition of ferric chloride and the same sample without addition of ferric chloride gave the concentration of desferrioxamine. In this manner desferrioxamine concentrations could also be measured in the presence of ferrioxamine. Plasma samples were deproteinised with trichloroacetic acid and analysed as above.

In the first experiments to be reported none of the several metabolites of desferrioxamine occurring in the blood was assumed to yield a coloured compound when treated with ferric iron. In the course of the experiments it was found, however, that one of the desferrioxamine metabolites occurring in the blood gives the same colour reaction with iron as desferrioxamine. This "metabolite C"[9] results from the oxidative desamination of the 1-amino-5-hydroxylamino-pentane residue No. 3 of the molecule (Fig. 1). The coloured iron complex is partially extracted into the benzyl alcohol while ferrioxamine is quantitatively extracted into this solvent. "Metabolite C" may be quantitatively eliminated by washing the benzyl alcohol extracts two or three times with a saturated solution of sodium bicarbonate. Fortunately, the renal clearance of "metabolite C" was found to be similar to that of desferrioxamine, so that data from earlier experiments were not invalidated.

In liquids like bile which contained coloured materials extractable into benzyl alcohol, desferrioxamine and ferrioxamine concentrations were estimated by extracting the ferrioxamine into benzyl alcohol and by measuring the iron concentration in the organic solvent. Fe determination was done by wet ashing in a mixture of sulphuric and nitric acid, decolouration by perhydrol, destruction of the excess of perhydrol by sodium sulphite, neutralisation with ammonia, reduction by hydroquinone, and colorimetric estimation of the ferrous iron with *o*-phenanthroline.

Regression lines, means and standard errors, and the significance of differences between means were calculated according to conventional statistical methods.[12] Numerical values from groups of observations are always given as mean $\pm$S.E.

RESULTS

Fate of desferrioxamine and of ferrioxamine in the nephrectomized dog

The logarithms of the concentrations of desferrioxamine found in the blood of 4 nephrectomized dogs after intravenous administration of a large dose fell on three different regression lines (Fig. 2). Of the first-order reactions described by these lines the first (first hour after t_0) was ascribed to equilibration in the final volume of distribution. At the end of this time inulin also had reached its final plasma concentration in all the experiments. A second first-order reaction occurred between the first and the fourth hour after the end of the infusion of desferrioxamine. It was interpreted as an expression of rapid metabolic degradation of desferrioxamine. Between the fourth and the eighth hour, plasma concentrations continued to fall, albeit at a much slower rate.

The volume of distribution of desferrioxamine was calculated by extrapolating the straight line describing the blood concentrations observed between the first and the fourth hour back to t_0, and by assuming that the total amount of desferrioxamine infused was present in the animals at t_0 (Table 1). If these assumptions are valid, desferrioxamine is distributed in a compartment which comprises total body water, or at

TABLE 1. VOLUME OF DISTRIBUTION OF DESFERRIOXAMINE AND OF INULIN IN FOUR NEPHRECTOMIZED DOGS

Dog	Weight (kg)	Total dose of desferrioxamine methanesulph. (expressed as free base) (mg/kg)	Total dose of inulin (mg/kg)	Desferrioxamine plasma concentration at t_0 (mg/100 ml)	Inulin plasma concentration 60–270 min after end of infusion	Volume of distribution % of body-wt.		"Biological half-life" ($t/2$) (min)
						Desferriox.	Inulin	
A ♂	15·0	309	52	60·9	28·3	50·7	18·4	104
B ♀	17·0	300	54	42·0	58·2	71·4	9·4	75
C ♂	16·0	154	56	20·0	49·4	77·1	11·4	57
D ♀	18·5	351	50	67·2	38·0	52·3	13·2	69
Mean						62·9	13·1	76
S.E.						±6·68	±2·13	±10·0

Data derived from analytical values shown in Fig. 2. Chemical estimation of plasma concentrations. In dog D "metabolite C" was eliminated before photometric measurements of the concentration (see methods). $t/2$ computed as T·ln 2, where T is defined by the relationship $\ln [\text{Desf.}]_t = \ln [\text{Desf.}]\, t_0 - \frac{t}{T}$.

least a very large fraction of total body water. The fairly low values for the volume of distribution of inulin measured simultaneously may be due to the liberation of histamine and to a consequent shift of water from the extracellular to the intracellular space.

The biological half-life of desferrioxamine in the nephrectomized dog, calculated from the second part of the curve describing the fall in plasma concentrations, was fairly short (76 $\pm$ 10 min).

In two experiments total radioactivity, i.e. the sum of the concentration of randomly labelled desferrioxamine and its metabolites, was also measured. Total radioactivity multiplied by the reciprocal value of the specific radioactivity of the drug injected measures the amount of a drug and its metabolites present in a given volume of a body fluid. The "radiochemical" concentrations of desferrioxamine metabolites in the blood of nephrectomized dogs fell much more slowly than the concentrations of desferrioxamine itself (Fig. 1). Degradation of the drug thus results in the accumulation of metabolites in the blood.

In contrast to desferrioxamine, its iron complex ferrioxamine did not appear to be degraded at any appreciable rate in the nephrectomized dog (Fig. 3). After the infusion of small or large doses and after equilibration the plasma concentrations of ferrioxamine declined very slowly. The fall could be described as a single first-order reaction.

The volume of distribution was slightly larger than the inulin space measured at the same time, and was compatible with a strictly extracellular distribution in the dog. (Table 2).

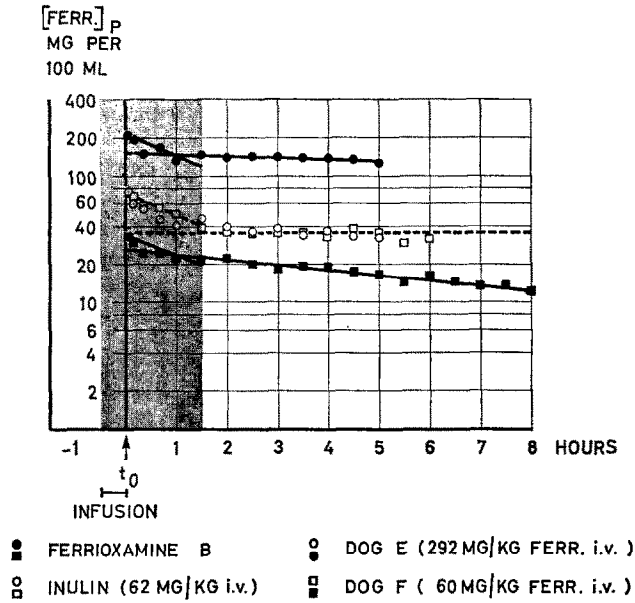

FIG. 3. Plasma concentrations of ferrioxamine B and of inulin in nephrectomized dogs after a large intravenous dose. Doses and plasma concentrations are expressed as ferrioxamine base. The uninterrupted lines show the plasma concentrations of ferrioxamine, and the broken lines the plasma concentrations of inulin.

TABLE 2. FERRIOXAMINE AND INULIN SPACES IN TWO NEPHRECTOMIZED DOGS

Dog	Weight (kg)	Total dose of ferrioxaminemethanesulphonate (expressed as free base) (mg/kg)	Total dose of inulin (mg/kg)	Plasma concentration of ferrioxamine at t_0 (mg %)	Plasma concentration of inulin 90–480 min after t_0	"Space" Percent of body-wt		"Biological half-life" ($t/2$) hr
						Ferrioxamine	Inulin	
E ♂	20	292	62	162·2	37·5	18·0	16·5	~18
F ♀	14	60	60	25·6	36·8	23·4	16·3	~7·6

Data computed from analytical values shown in Fig. 3. $t/2$ defined and computed as in Table 1.

Renal clearance of desferrioxamine and ferrioxamine in the dog

The renal clearance of desferrioxamine (C_{Desf}) was measured in 6 dogs (Fig. 4) at different plasma concentrations. In 5 animals it was lower than the glomerular filtration rate (clearance of inulin = C_{In}) at plasma levels below 10 mg per cent of desferrioxamine, and tended to approach C_{In} at higher plasma levels. These data suggest excretion by glomerular filtration and the presence of a tubular reabsorptive mechanism with a low transport capacity (*Tm*). In one dog (dog 1, Fig. 4) there was, however, evidence of the presence of a secretory mechanism (Fig. 4, Table 3). In this animal the clearance of PAH (C_{PAH}) was rather low. No competition for a common secretory mechanism between desferrioxamine and PAH can, however, be construed from the low values of C_{PAH}, since the filtration fractions were within the normal range.

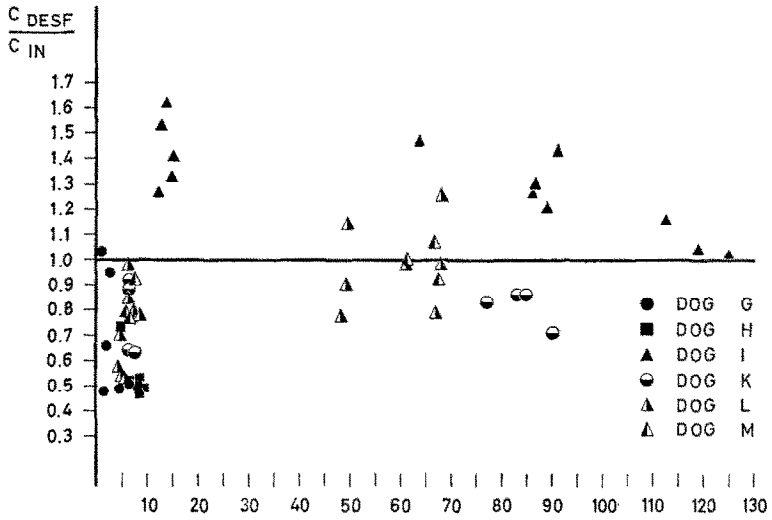

FIG. 4. Influence of plasma concentrations of desferrioxamine on C_{Desf}/C_{In} in six anaesthetized dogs. Each point represents the mean of two to four successive clearance periods of 3–25 min duration. The values for dogs G and H, and partly also for dog L, were measured during isotonic saline diuresis. All other values were measured during mannitol diuresis.

In 4 of these 6 dogs, desferrioxamine concentrations in the plasma and urine were measured as "total desferrioxamine", i.e. as desferrioxamine + metabolite C. In 2 animals (Table 4 vs. Table 3) the renal clearances of desferrioxamine and of metabolite C were measured separately at two different rates of infusion of desferrioxamine. The clearances for both substances were very similar. Measurements in which the presence of metabolite C was disregarded may, therefore, be considered as valid.

The renal clearance of desferrioxamine also depended on urine flow (Fig. 5). C_{Desf} tended to be lower than C_{In} at low rates of urine flow. In addition to a slight degree of tubular reabsorption, some desferrioxamine may thus also reach the blood stream from tubular fluid by diffusion from the terminal parts of the nephrons where the drug reaches high concentrations.

The renal excretion pattern of ferrioxamine in the dog was comparable to that of desferrioxamine (Fig. 6, Table 5). While at very low plasma concentrations there was definite evidence of tubular reabsorption, the clearance of desferrioxamine tended to

TABLE 3. RENAL CLEARANCE OF DESFERRIOXAMINE IN DOGS ANAESTHETIZED WITH PENTOBARBITONE

Dog	Weight (kg)	Infusion	Periods: ml / kg min	Periods: duration (min)	Periods: number	Urine flow (ml/kg.min)	Desferrioxamine in plasma (mg%)	C_{Dest} (ml/kg.min)	C_{In} (ml/kg.min)	C_{PAH} (ml/kg.min)	$\frac{C_{Dest}}{C_{In}}$	$\frac{Extr_{Dest}}{Extr_{In}}$
G ♂	21.0	0·9% NaCl	0·05	27–32	6	0·011	(Total) 1·1	(Total) 2·3	3·7	9·6	0·66	
		0·9% NaCl	0·05	30	4	0·011	(Total) 5·3	(Total) 1·8	2·7	9·4	0·71	
H ♂	28·0	0 9% NaCl	0 04	22–42	8	0 020	(Total) 8·4	(Total) 2·3	3·6	9·3	0·59	
I ♂	27·0	10% mannitol +	0·156	5–6	6	0·105	(Total) 13·0	(Total) 1·9	1·3	3·4	1·44	1·38
		0·9% NaCl		5–10	6	0·055	(Total) 81·6	(Total) 0·56	0·44	1·6	1·30	1·27
K ♀	16·0	10% mannitol +	0·313	2–3	8	0·272	(Total) 6·0	(Total) 2·4	2·9	18·3	0·82	
		0·9% NaCl		3–4	8	0·216	(Total) 84·7	(Total) 1·5	2·0	9·2	0·78	
L ♂	19·0	0·9% NaCl	0·263	5	10	0·048	(True) 4·8	(True) 2·2	4·9	8·3	0·50	
		10% mannitol +	0·263	4–5	7	0·139	(True) 5·0	(True) 1·7	2·8	5·8	0·63	
		0·9% NaCl	0·263	3–3·5	7	0·183	(True) 48·7	(True) 2·0	2·1	6·6	0·94	
M ♀	13·8	10% mannitol +	0·362	3–4	8	0·502	(True) 7·0	(True) 2·2	2·7	9·9	0·83	
		0·9% NaCl		3–4	8	0·405	(True) 65·1	(True) 2·0	2·1	4·2	0·99	

Clearances measured simultaneously in dogs anaesthetized with pentobarbitone. Values are means from a specified number of collecting periods, in the course of which neither the rate of administration of desferrioxamine or the rate of infusion, nor the urine flow or blood pressure varied significantly. In dogs G–K the concentrations of desferrioxamine measured in the plasma and urine were those of desferrioxamine + metabolite C. In dogs L and M true desferrioxamine measured in the plasma and urine were those of desferrioxamine + metabolite C. In dogs L and M true desferrioxamine and metabolite C were estimated separately. In dog I renal extraction of desferrioxamine and of inulin was also measured.

TABLE 4. RENAL CLEARANCE OF "METABOLITE C" IN TWO DOGS INFUSED WITH DESFERRIOXAMINE B

Dog	Weight (kg)	Infusion	Periods			Urine flow (ml/kg.min)	Metabolite C in plasma (expressed as desferrioxamine mg %)	$C_{metabolite}$ (ml/kg.min)	C_{In} (ml/kg.min)	C_{PAH} (ml/kg.min)	$\frac{C_{metabolite}}{C_{In}}$
			ml / kg min	duration (min)	number						
L ♂	19 0	0 9% NaCl	0·263	5	10	0·048	Metabl. C 2·3	2·7	4·9	8·3	0·52
		10% mannitol +	0·263	4–5	7	0.139	Metab. C 2·5	2·2	2·8	5·8	0·81
		0 9% NaCl	0·263	3–3·5	7	0·139	Metab. C 15·6	1·9	2·1	6·6	0·89
M ♀	13 8	10% mannitol +	0·362	3–4	8	0·502	Metab. C 4·1	2·4	2·7	9·9	0·90
		0 9% NaCl		3–4	8	0·405	Metab. C 28·8	1·6	2·1	4·2	0·79

exceed C_{In} at plasma concentrations between 8 and 16 mg per cent and to approach C_{In} at higher plasma concentrations. There was no evidence of the occurrence of diffusional losses of ferrioxamine from tubular fluid at low rates of urine flow (Table 5).

Renal excretion of desferrioxamine and of ferrioxamine in unanaesthetized rats

In the rat C_{Desf} was much higher than C_{In} at plasma desferrioxamine levels below

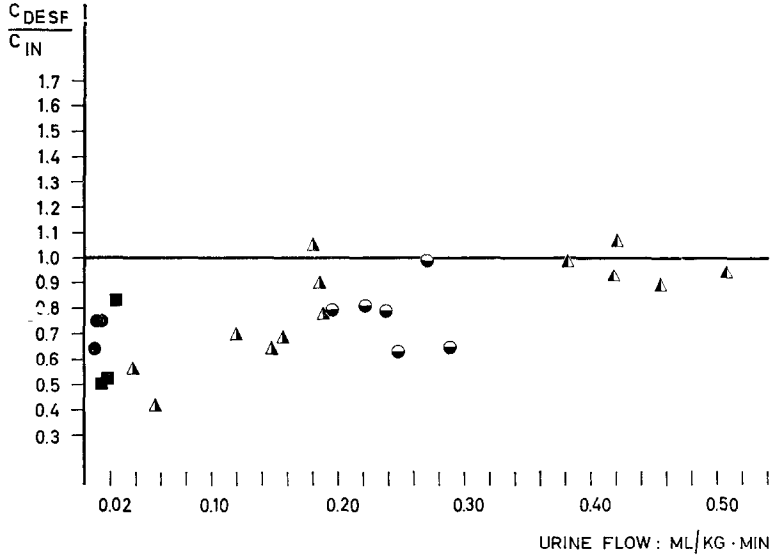

FIG. 5. Changes in C_{Desf}/C_{In} with varying rates of urine flow in five dogs. Each point represents the mean for two to four successive clearance periods. The symbols for each animal are the same as in Fig. 4. The one dog which showed evidence of tubular secretion of desferrioxamine (dog 1) is not included in this graph.

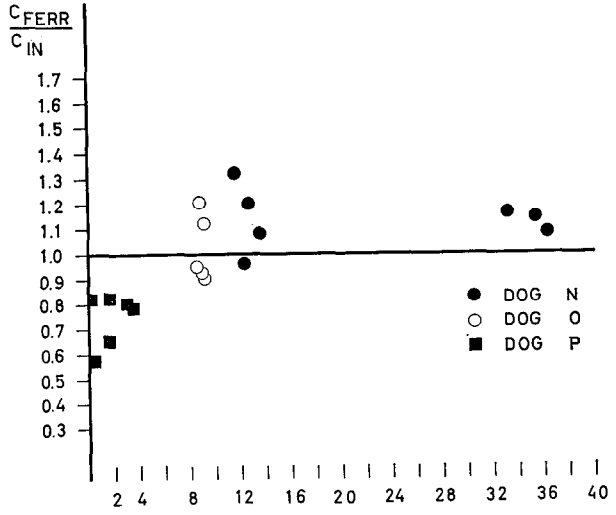

FIG. 6. C_{Ferr}/C_{In} at low and at medium plasma levels of ferrioxamine in dogs. Each point represents the mean of 2–3 successive clearance periods. The different symbols indicate different animals.

TABLE 5. RENAL CLEARANCE OF FERRIOXAMINE IN DOGS

Dog	Weight (kg)	Infusion	Periods: ml / kg min	Periods: Duration (min)	Periods: Number	Urine flow (ml/kg.min)	[Ferr.] in Plasma mg %	$C_{Ferr.}$ (ml/kg.min)	C_{In} (ml/kg.min)	C_{PAH} (ml/kg.min)	$\frac{C_{Ferr.}}{C_{In}}$
N ♂	18·0	0·9% NaCl	0·208	12–16	5	0·024	12·6	4·2	4·3	7·7	1·04
		12% mannitol +	0·208	6–8	6	0·125	12·6	3·6	2·9	7·0	1·22
		0·9% NaCl	0·208	5–9	8	0·176	34·8	3·3	2·9	8·7	1·14
O ♀	14·0	0·9% NaCl	0·268	6–10	5	0·098	8·8	4·8	5·2	13·5	0·93
		10% mannitol + 0·9% NaCl	0·268	3–4·5	7	0·259	8·9	4·7	4·5	10·3	1·05
P ♂	19·0	10% mannitol +	0·263	3–4·5	6	0·232	0·39	4·8	6·9	21·3	0·70
		0·9% NaCl	0·263	4–4·5	6	0·196	1·63	5·4	7·8	14·8	0·71
				4–5	5	0·155	3·13	6·2	7·8	12·3	0·80

Clearances were measured simultaneously in dogs anaesthetized with pentobarbitone. The values given are means from a specified number of successive periods in which neither plasma levels and clearance of ferrioxamine, nor other parameters of renal function, nor arterial blood pressure changed significantly.

30 mg per cent. At higher plasma concentrations C_{Desf} was equal to C_{In} (Fig. 7). These data demonstrate tubular secretion of desferrioxamine. C_{PAH} was not significantly depressed even at high plasma concentrations of desferrioxamine. There was no evidence of tubular reabsorption of desferrioxamine at any plasma level.

Ferrioxamine, on the other hand, was reabsorbed from tubular urine at low plasma levels both in the rat and in the dog (Fig. 8). At plasma levels above 10 mg per cent the renal clearance of ferrioxamine became equal to the glomerular filtration rate. At very high plasma concentrations ferrioxamine depressed C_{PAH}.

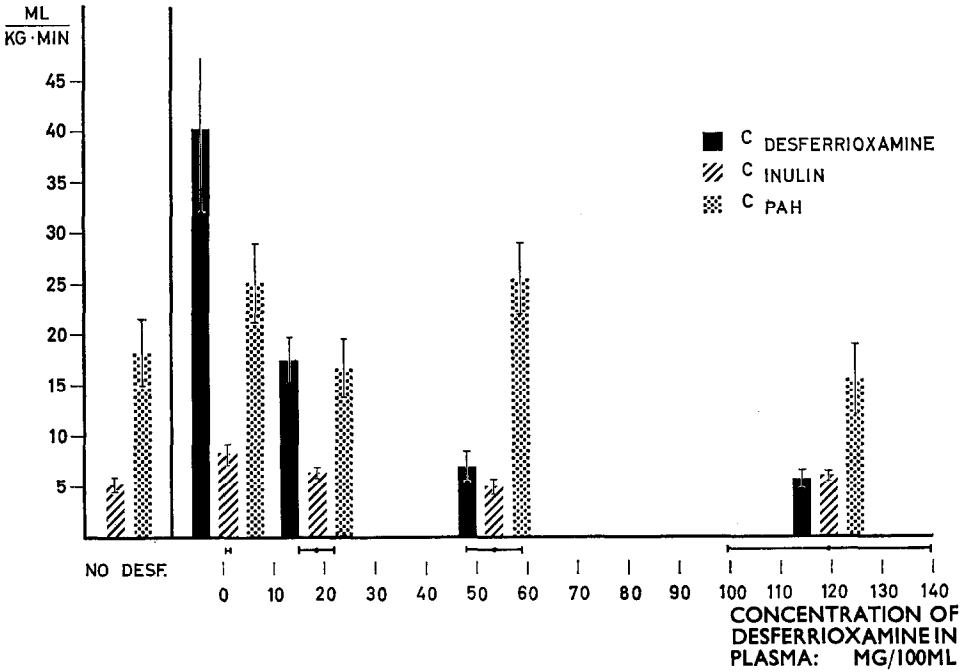

FIG. 7. Renal clearance of desferrioxamine, inulin, and PAH at different plasma levels of desferrioxamine in unanaesthetized rats. GFR, C_{PAH} and C_{Desf} measured simultaneously in groups of 8 to 15 animals infused with isotonic saline. The clearances are given as means (columns) ± S.E. indicated by vertical lines with brackets. The plasma concentrations are given as means ± S.E. (horizontal lines with brackets.)

Desferrioxamine and ferrioxamine in other body fluids

Traces of desferrioxamine were found in the cerebrospinal fluid in two nephrectomized dogs at a time when the plasma levels had declined to 13 and 12 mg per cent respectively. The CSF did not contain any ferrioxamine in one nephrectomized dog at a plasma concentration above 150 mg per cent.

In two nephrectomized dogs infused with desferrioxamine and tritium-labelled desferrioxamine, the bladder bile at the end of an experiment was very rich in radioactive desferrioxamine metabolites, but did not contain any appreciable amounts of unaltered desferrioxamine.

In dogs infused with ferrioxamine the sideramine was found in slight amounts in the bladder bile.

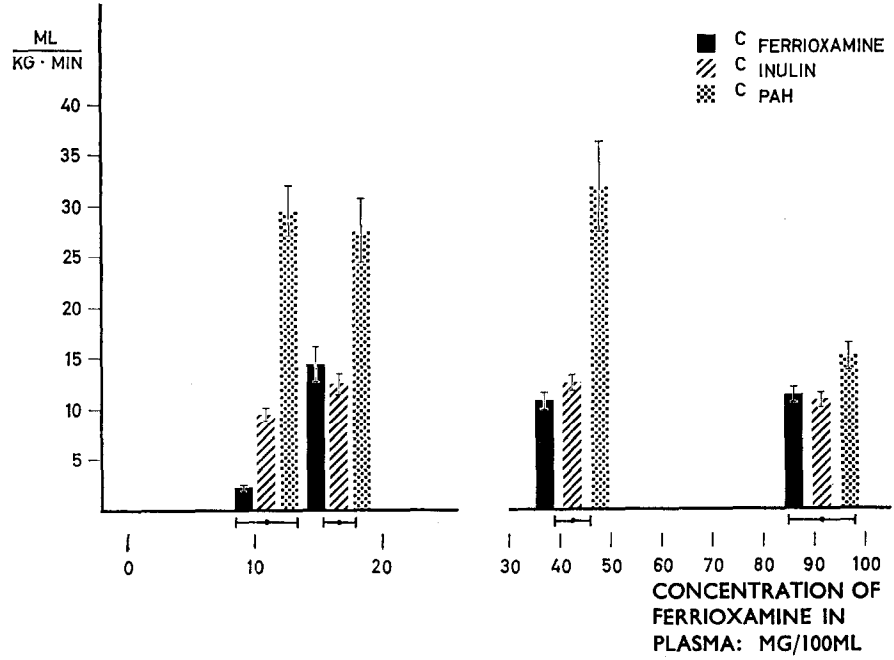

FIG. 8. Renal clearance of ferrioxamine at different plasma concentrations in unanaesthetized rats. Conditions as in the desferrioxamine experiments summarised in Fig. 7. Each group of three columns gives mean values (columns) ± S.E. (for groups of 9–15 rats). The plasma concentrations are indicated as in Fig. 7.

DISCUSSION

The rapid fall in the plasma concentrations of desferrioxamine in intact animals appears to be due not only to rapid renal excretion, but also to rapid metabolic destruction. If the rate of equilibration of desferrioxamine in its final volume of distribution is assumed to be the same as that of inulin, the rate of destruction may be expressed by two first-order reactions. After a rapid fall of plasma concentrations during the first few hours there is a much slower decline in the plasma concentrations between the fourth and the eighth hour. The steep fall in the plasma concentration between the first and fourth hour after a large intravenous dose is due to metabolic destruction of desferrioxamine. The slow decline in the desferrioxamine plasma concentrations between the fourth and eighth hour is due to the metabolic destruction of a desferrioxamine metabolite which forms coloured iron complexes, and which may be identical with metabolite C.

The distribution volumes of desferrioxamine in nephrectomized dogs were calculated on the assumption that two separate first-order reactions describe the process of metabolic degradation. If the concentration in the plasma at t_0 were obtained by extrapolation from the common regression line of all values observed within eight hours, the resulting unrealistic volume of distribution would be greater than the total body weight. Separating the two periods at four hours after t_0 may, however, result in an over-estimation of the plasma concentration at t_0, and thus in an under-estimation of the volume of distribution. The volume of distribution, on the other hand, is clearly over-estimated by assuming that the whole infused dose was present in the body of the experimental animals at t_0, since some metabolic destruction must have occurred during

the period of infusion. This source of error could not be avoided, since a more rapid injection of high doses of desferrioxamine causes irreversible shock in dogs.[11] The volumes of distribution for desferrioxamine must thus be accepted as approximate figures. Allowing for all possible uncertainties it may, however, be stated with confidence that desferrioxamine is distributed in a volume which is definitely larger than the extracellular space.

The large volume of distribution of desferrioxamine cannot, however, be construed to mean that the compound penetrates into the intracellular compartment of all tissues. An accumulation of the compound in the cells of some tissues, as for instance the liver, would result in similarly large volumes of distribution.

The stability of ferrioxamine in the body of the nephrectomized animal contrasts sharply with the rapid destruction of the iron-free compound. Besides the fact that it changed its physico-chemical properties, the binding of trivalent iron thus also appears to protect the molecule against enzymatic degradation.

This protection recalls the greater resistance of ferritin to an enzymatic hydrolysis as compared with apoferritin.[18]

Biliary excretion may account for the slow decline in the ferrioxamine plasma concentrations in the nephrectomized animal. The volume of distribution of ferrioxamine in the nephrectomized dog is clearly somewhat larger than the volume of distribution of inulin. It may be equated with the extra-cellular space, as measured, for instance, by sucrose and other "extracellular" substances with a diffusibility higher than that of inulin.

The rapid renal excretion of desferrioxamine in the dog appears to be due mainly to glomerular filtration and a reabsorptive mechanism with a low transport capacity. Since only one dog was found to secrete desferrioxamine at the tubular level, there may be differences between different strains of dogs in this respect. In the rat, active tubular secretion of desferrioxamine could be clearly demonstrated. There are thus, species as well as strain, differences. The tubular secretory mechanism for desferrioxamine in the rat does not appear to be identical with the transport mechanism for PAH.

When injected at doses which do not induce shock, neither desferrioxamine nor ferrioxamine interfere with the renal functions of rats or dogs.[19]

While the rapid renal excretion of desferrioxamine may be a drawback as regards the therapeutic use of this drug for removing iron deposits, the equally high rate of renal excretion of ferrioxamine appears to be a desirable feature.

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