Table 1. Lattice spacing (Å) (±0·05 Å) of sulphathiazole I, $C_9H_9N_3O_3S_2$.

Temperature of crystallization		
0°C	30°C	70°C
7·24	—	7·94
3·40	5·83	5·47
2·43	2·92	4·08
2·29	2·43	2·84
2·09	2·03	2·73
1·48	1·46	2·32
	1·26	1·96
	1·20	1·70
	1·03	1·46
	0·93	1·19
	0·86	1·05
	0·63	0·94

regions of discontinuity in every specimen examined. The regions of discontinuity showed lattice distortions near them and therefore it is thought that these regions are connected with the presence of dislocations. In particular, we can refer to Fig. 5, where a dislocation was seen due to favourable specimen orientations in relation to the electron beam.

Table 1 gives lattice spacings obtained in this study from selected area diffraction patterns. Reflections corresponding to the large lattice spacings could be missing due to strong (000) reflections.

There is considerable confusion in the literature concerning the number and status of the polymorphic forms of sulphathiazole and this is compounded by the use of different nomenclatures for the different forms (Moustaffa & Carless 1969; Kruger & Gafner 1972; ASTM Data). A mixture of forms II and III of Kruger & Gafner, on DSC, would resemble form I of this study and the presence of such mixtures may be difficult to confirm if one form is present in small quantities. The results given in Table 1 are of form I. The sulphathiazole crystals examined were very small, less than 1000 nm. It is probable that much larger crystals are required if the defects observed with potassium perchlorate (Burt & Mitchell 1981) are to be observed.

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Pharmacokinetic study of tempo carboxylic acid, a nitroxyl MRI contrast media, in control and streptozocin diabetic rats

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Abstract—The pharmacokinetics of tempo carboxylic acid (TCA), a nitroxyl contrast medium have been evaluated in control and streptozocin-diabetic rats. Previous magnetic resonance imaging (MRI) studies in diabetic rats showed prolongation of contrast visualization in the renal cavities after injection of TCA. Diabetes induced only slight alterations to the pharmacokinetic parameters. Rate constants and half lives were unchanged after four months of diabetes. A significant decrease of the apparent total body clearance and volume of distribution was observed while the area under the curve was increased. These alterations are not sufficient to explain MRI abnormalities which have to be elucidated.

Tempo carboxylic acid (TCA) is a piperidinic nitroxide spin label (nitroxide stable free radical) which has been successfully used as an experimental contrast medium for magnetic resonance imaging (MRI). TCA, as a meglumine salt, presented a renal elimination (Lamarque et al 1986) and has been used to assess renal function in normal rats and rats with experimentally induced ischaemia, hydronephrosis (Lamarque et al 1986) and diabetic nephropathy (Bonnet et al 1987; Chapat et al 1987). MRI studies in diabetic rats showed prolongation of contrast visualization in the renal cavities (45 min in diabetic rats versus 15 min in control rats) which was not clearly understood. The purpose of this study was to evaluate pharmacokinetics parameters of TCA after intravenous (i.v.) administration in control and diabetic rats to specify the causal factor responsible for MRI abnormalities.

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Material and methods

Induction of diabetes. Male Wistar rats, 210–230 g were randomly selected for this study and maintained on ordinary rat chow and water without restriction. Diabetes was induced, under light ether anaesthesia, by a single i.v. dose of streptozocin (STZ, Sigma Co., USA) 50 mg kg^{-1} administered in 1 mL kg^{-1} citrate buffer 0·05 M pH 4·5 in which it was dissolved just before injection. That dose of streptozocin is known to produce a mild diabetic state with hyperglycaemia but without ketosis (Tancrede et al 1983). Control rats received an equivalent volume of citrate buffer. Seven days after the onset of diabetes, plasma glucose was determined in STZ-treated rats (Destrostix-Ames Co.) and rats with glucose levels lower than 250 mg% were discarded. Diabetic state was controlled by taking into account the following parameters: water intake, food intake and body weight. Plasma glucose and urine volume were measured at the end of the study.

Pharmacokinetic studies. This study was made four months after induction of diabetes. Six control rats and 6 diabetic rats were anaesthetized with intraperitoneal ethylcarbamate (1·2 g kg^{-1}). TCA meglumine (2 mM kg^{-1}) was injected into a tail vein in a volume of 3 mL kg^{-1}. Blood samples (300 μL) were taken 1·5, 3, 5, 10, 15, 20, 30, 45, 60 and 75 min after injection, via a heparinized carotid catheter. Blood nitroxide concentrations were determined at room temperature (20°C) by electron paramagnetic resonance (EPR) using a Brucker ER200D spectrometer. The concentration of paramagnetic radical was determined after double integration of the nitroxide EPR triplet. A direct correlation ($y=0{\cdot}89x-0{\cdot}56$ $R=0{\cdot}99$) between the three line signal double integration and nitroxide concentration was previously assessed using increasing concentrations of TCA-meglumine in rat blood samples. Instrument settings for data collection were 3370G central magnetic field, 1G field modulation intensity, 0·2s time constant, 100G scan range, 200s scan time. Blood samples were heparinized and assayed immediately for nitroxide concentration. A primary study showed this to be stable within 1 h after sample collection at the room temperature. Pharmacokinetic parameters were calculated from the plasma values with a computer program using the method of residuals and linear least square regression analysis (Cazin & Luyckx 1984).

Statistics. Comparison between control and diabetic groups were made using the non parametric Mann Whitney test. The level of significance was set at $P<0{\cdot}05$.

Results and discussion

Diabetes. Several parameters indicating the general condition of the two groups of animals are given in Table 1. The data indicate a significant increase of plasma glucose, urine volume, water and food intake for diabetic rats while for these animals a decreased body weight was noted.

Table 1. Effect of diabetes on body weight, urine volume, water and food intake, and plasma glucose. Numbers are mean ± s.e.m of the values obtained from 6 animals in each group.

	Control	Diabetic
Body weight (g)	588·0 ± 17	343·0 ± 20*
Plasma glucose (g L^{-1})	1·1 ± 0·02	3·4 ± 0·35*
Urine volume (mL day^{-1})	7·3 ± 0·5	235·4 ± 24·6*
Water intake (mL day^{-1})	37·0 ± 4	280·0 ± 25*
Food intake (g day^{-1})	36·0 ± 3	56·0 ± 8*

* Mann Whitney test: values significantly different from control ($P<0{\cdot}05$)

Pharmacokinetic studies. The behaviour of TCA meglumine after i.v. administration can be described as an open two compartment model both in control and diabetic rats. This is assessed by the biexponential decay of the blood nitroxide concentration with time (Fig. 1). These curves showed that there are similar patterns to the TCA blood levels in control and diabetic rats but TCA was higher in diabetic rats (Table 2).

Table 3 indicates that TCA was rapidly eliminated after a short distribution phase. As with other nitroxide components (Griffeth et al 1984), TCA is mainly confined to the vascular and other extracellular aqueous compartments. Diabetes induced only slight alterations in the pharmacokinetic parameters. Disposition rate constants (α, β), distribution rate constants (K_{12}, K_{21}), elimination rate constant (K_{el}) and half lives were unchanged after four months of diabetes. On the contrary, a

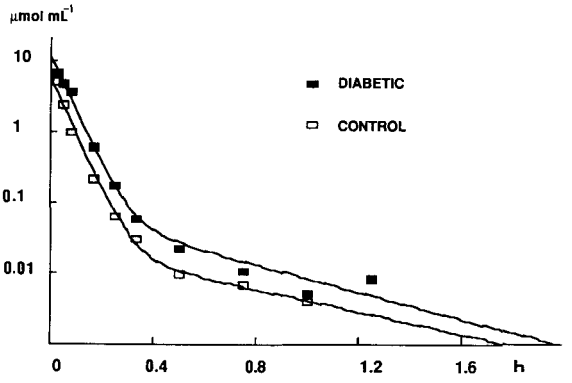

FIG. 1. Semi-logarithmic plots of blood TCA concentration versus time in control and diabetic rats.

Table 2. Effect of diabetes TCA blood levels (μmol L^{-1}). Numbers are mean ± s.e.m of the values obtained from 6 animals in each group.

Time (min)	Control	Diabetic
1·5	5518·3 ± 563·9	8662·3 ± 1004·3*
3	2268·3 ± 260·5	3875·6 ± 545·3
5	1052·7 ± 144·5	1967·0 ± 364·5*
10	281·8 ± 67·9	494·5 ± 94·3
15	110·7 ± 28·9	132·9 ± 22·2
20	51·7 ± 13·6	74·9 ± 12·3
30	17·9 ± 4·2	23·6 ± 4·2
45	9·3 ± 1·4	10·4 ± 2·5
60	5·4 ± 0·7	8·9 ± 0·7*
75	—	8·1 ± 0·7

* Mann Whitney test: values significantly different from control ($P<0{\cdot}05$)

Table 3. Pharmacokinetic parameters of TCA after i.v. administration in control and diabetic rats. Numbers are mean ± s.e.m of the values obtained from 6 animals in each group.

Pharmacokinetic parameters	Control	Diabetic
α (min^{-1})	0·29 ± 0·01	0·32 ± 0·04
β (min^{-1})	0·04 ± 0·01	0·03 ± 0·00
T1/2 α (min)	2·41 ± 0·12	2·26 ± 0·25
T1/2 β (min)	20·11 ± 2·31	24·22 ± 3·31
K_{12} (min^{-1})	0·015 ± 0·000	0·017 ± 0·00
K_{21} (min^{-1})	0·039 ± 0·005	0·033 ± 0·000
K_{el} (min^{-1})	0·274 ± 0·015	0·177 ± 0·024
Volume of distribution (L kg^{-1})	0·33 ± 0·03	0·18 ± 0·00*
Total body clearance (mL kg^{-1} min^{-1})	90·60 ± 11·5	51·71 ± 4·41*
AUC (μmol mL^{-1} h)	18·90 ± 2·5	30·30 ± 2·2*

* Mann Whitney test: values significantly different from control ($P<0{\cdot}05$)

significant decrease of the apparent total body clearance and volume of distribution was observed while the area under the curve (AUC) was increased after four months of diabetes.

Some differences between human and rat diabetic renal disease have been reported. In rats, the earliest and major alteration consists in epithelial degenerative changes of the distal tubule whereas the proximal tubule is involved in man (Bleasel & Young 1982). Morphological changes in glomerular structures occurred in long-term diabetic rats similar to those seen in human diabetes (Taylor et al 1980). All these alterations seem to have little influence on TCA elimination since rate constants are unchanged in diabetic rats. As diabetic rats failed to gain weight, the muscular loss explains the decrease of the volume of distribution and consequently the increase of TCA blood levels

and the increase of AUC. The decrease of the total clearance can be, in part, attributed to the decrease in the volume of distribution. But the total clearance comprises not only renal excretion but all other pathways of elimination including drug metabolism. Diabetes mellitus involves disorders of many metabolic pathways. The hypothesis of a diabetic-induced decrease of the nitroxide reduction cannot be discarded.

In summary, the initial increase of blood concentration of free radical is insufficient to explain the MRI abnormalities observed since elimination rates are rapid and similar both in control and diabetic rats. The prolongation of contrast visualization in the kidney might be explained by a stasis of contrast media in the kidney secondary to the tubular nephropathy and, or, a decrease of renal metabolism leading to a persistance of paramagnetism.

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The protective mechanisms of paracetamol against ethanol-induced gastric mucosal damage in rats

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Abstract—The protective mechanisms of paracetamol against ethanol-induced gastric mucosal damage have been examined. The antiulcer action of subcutaneously (s.c.)-injected paracetamol, 250 mg kg^{-1}, was attenuated by either subdiaphragmatic vagotomy or s.c. injection of *N*-ethylmaleimide, 10 mg kg^{-1}. This attenuation was not seen in rats given paracetamol by the oral route (p.o.). Indomethacin pretreatment, 5 mg kg^{-1}, did not influence the lesion-preventing action of paracetamol given s.c. or p.o. The findings suggest that the antiulcer effect of s.c.-administered paracetamol results from an action involving the vagal nerve and tissue sulfhydryls, but not prostaglandins. On the other hand, the protective mechanism of paracetamol p.o. is independent of the vagal system or tissue sulfhydryls and prostaglandins. It seems that paracetamol given p.o. exerts its antiulcer effect by acting directly on the mucosal cell to strengthen mucosal integrity.

Paracetamol when given orally (p.o.) reduces ethanol-induced gastric mucosal damage (Poon et al 1988). As subcutaneously (s.c.)-injected paracetamol also effectively antagonizes ethanol-induced gastric lesions (Poon et al 1988), it is possible that the protective mechanism could be mediated, partly or wholly, through a systemic pathway. Somasundaram & Ganguly (1987) have shown the importance of the vagus nerve in maintaining normal levels of gastric mucus glycoproteins and, thus, mucosal integrity; absence of vagal influence weakens the mucus barrier. It is, therefore, reasonable to postulate that the systemic action of paracetamol may be mediated through the vagal nerve.

Sulfhydryls and prostaglandins protect against ethanol-induced mucosal damage (Szabo 1986; Konturek et al 1987a). As paracetamol has been shown to stimulate prostaglandin production in the gastric wall (Van Kolfschoten & Van Noordwijk 1987), it has been suggested that these eicosanoids may participate in the lesion-antagonizing mechanism of the drug. The role of sulfhydryls and prostaglandins in the antiulcer action of paracetamol is, however, still unclear.

The present study examines the role of the vagus, sulfhydryls and prostaglandins in relation to the protective action of paracetamol against ethanol-induced gastric mucosal damage in rats.

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Materials and methods

Male Sprague-Dawley rats were fed a standard laboratory pellet diet (Ralston Purina Co.) and drank tap water. Food was withheld 24 or 48 h before the animals were used, depending on the type of experiment; the rats were kept in cages with wide wire mesh floors to prevent coprophagy and allowed free access to tap water which was removed 1 h before starting experiments. All experiments were conducted in an air-conditioned room (temperature 22 ± 1°C, relative humidity 65–70%) where the animals were normally housed. The rats were killed, by a sharp blow on the head, immediately at the end of each experiment and their stomachs removed. The areas of the lesions in the glandular segment of the stomach were measured with a grid (each grid was 1 mm^2) (Ogle et al 1985). In the case of petechiae, five such lesions were taken as the equivalent of 1 mm^2. The sum of the lesion areas in each group was divided by the number of animals and expressed as the mean lesion index.

Experiment 1. Rats (180 ± 20 g) were subjected to subdiaphragmatic vagotomy under light ether anaesthesia. The upper abdominal cavity was opened by a midline incision beneath the sternum. The anterior and posterior branches of the vagal nerve,