Many redox steps are involved in the metabolism of proline. The oxidation of proline is NAD-dependent but, in the reduction of glutamate to proline, NADP-dependent dehydrogenases are involved. Since the NAD-dependent reactions are more affected by ethanol than the NADP-dependent reactions [23], the oxidation of proline is thus in all probability inhibited more than its formation is promoted [24].

In summary, a single dose of ethanol caused a significant but temporary increase in the concentration of both proline and glutamate in the rat liver, maximally + 37 and + 149 per cent, respectively. The concentrations of glutamate and proline had a linear correlation. When 4-methylpyrazole was given simultaneously with ethanol there were no changes in the concentrations of proline and glutamate. The administration of methylene blue corroborated the effects of ethanol.

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Inhibition of hepatic metabolism of azathioprine by furosemide in human liver *in vitro*

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The immunosuppressive drug azathioprine undergoes thiolysis to 6-mercaptopurine, a biotransformation important for the ultimate action of the drug [1, 2]. This conversion is catalyzed by glutathione (GSH) S-transferase [3] predominantly in the liver [4]. *In vivo* studies in rats have shown inhibition of hepatic metabolism of azathioprine by endogenous and exogenous glutathione S-transferase inhibitors, bilirubin and probenecid, respectively [5]. After kidney transplantation, azathioprine is often used together with the diuretic drug furosemide, which can inhibit GSH S-transferase activity in the kidney [6]. Since the liver seems to play the dominant role in the glutathione mediated metabolism of azathioprine [4], it was of interest to study whether furosemide could also inhibit the formation of 6-mercaptopurine in this tissue. Such an interaction could possibly result in a diminished immunosuppressive effect of azathioprine. Therefore we have studied furosemide inhibition of the formation of 6-mercaptopurine in the liver *in vitro*. We used human liver from our human liver bank [7] since marked species differences in the metabolism of xenobiotics exist.

Eight specimens of human adult liver were obtained within 20 min after stopping artificial respiration and life supporting treatment of patients without cerebral activity who were selected as kidney donors. Homogenization and subcellular fractionation was initiated shortly thereafter, as described elsewhere [7]. The 100,000 *g* supernatant fraction was stored at −80° until used. Relevant patient data are depicted in Table 1. Some donors received drugs the last few days before death. However, usually they received only single (e.g. during anesthesia) or a few doses, and we believe that this pre-mortem treatment did not markedly influence the capacity of the liver to metabolize drugs.

The GSH-S-transferase activity with azathioprine in the 100,000 *g* liver 'cytosol' fractions was determined according to methods previously described [3]. The final reaction mixture (3.0 ml) consisted of azathioprine (0.1 or 0.2 mM) dissolved in 0.1 M sodium phosphate buffer, pH 6.5, 0.17 mM reduced glutathione and 200 μl of liver cytosol. The reaction was measured by the production of the 317 nm product (6-mercaptopurine) in a Beckman ACTA MVI spectrophotometer at 37° with the reference containing

Table 1. Clinical data of liver donors

Sample	Sex	Age	Cause of death/disease	Relevent known drug treatment
1	M	27	Trauma Cerebral concussion	Few doses of betamethasone and diazepam
2	F	69	Cerebral infarction	Single doses of dexamethasone and pethidine
3	F	52	Meningioma Post-operative cerebral infarction	Few doses of furosemide, paracetamol, dihydroergotamin and metoclopramide
4	F	59	Cerebral aneurysm	
5	M	49	Pontine haemorrhage Epilepsy	Single doses of lidocaine and dexamethasone, possibly antiepileptics
6	M	18	Road accident Cerebral concussion	Single dose of dexamethasone
7	M	25	Suicide Multiple trauma	Repeated doses of furosemide and diazepam
8	F	53	Cerebral aneurysm	Repeated doses of phenytoin, diazepam and pentazocine; few doses of aminocaproic acid and paracetamol

Table 2. Inhibition of enzymatic thiolysis of azathioprine by furosemide

Liver sample No.	Transferase specific activity with azathioprine* (nmoles/min/mg)	Apparent K_i (mM)
1	2.90	0.10
2	2.75	0.53
3	7.59	0.10
4	6.57	0.13
5	4.41	0.30
6	1.57	0.15
7	3.15	0.04
8	5.33	0.23
Mean	4.28	0.23

* Specific activity determined with 0.1 mM azathioprine.

azathioprine dissolved in buffer. The final value for enzyme activity was obtained by subtracting nonenzymatic product formation in the presence of reduced glutathione and azathioprine from the total activity of a given reaction. The nonenzymatic reaction was usually less than 10 per cent of total activity under these conditions. The enzymic reactions were found to be linear with respect to time and substrate concentration.

Inhibitory kinetics in the presence of furosemide were determined by using two different azathioprine concentrations and a range of furosemide concentrations. The data obtained were expressed by the method of Dixon [8] and the inhibitor constants, representing the value on the abscissa corresponding to the intersection of the two lines, were calculated from the corresponding least squares regression equations.

Azathioprine was obtained from Burroughs, Wellcome & Co., Research Triangle Park, NC. Furosemide was purchased from Hoescht Pharmaceuticals, Somerville, NJ and reduced glutathione from the Sigma Chemical Company, St. Louis, MO.

Protein concentration was determined according to the Lowry method [9] using bovine serum albumin (Sigma) as the standard.

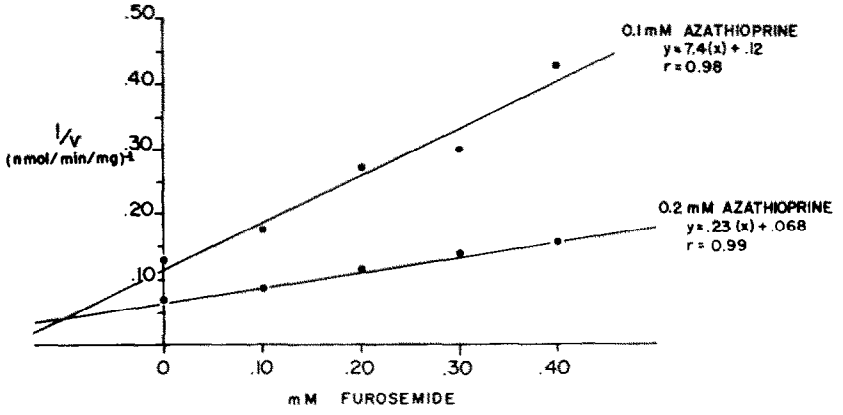

Fig. 1. Dixon plot of the competitive inhibition by furosemide of the enzymatic thiolysis of the azathioprine catalyzed by the glutathione S-transferases.

All eight samples of human liver cytosol were found to catalyze the enzymatic thiolysis of azathioprine with glutathione (Table 2). The inhibitory kinetic data obtained in the presence of furosemide was examined using a Dixon plot analysis. Figure 1 is a representative example obtained with sample 3 and demonstrates that furosemide is a competitive inhibitor of the GSH-S-transferase mediated thiolysis of azathioprine. The apparent K_i for the inhibition of this reaction by furosemide was found to be 0.10 mM. Furosemide was found to be a competitive inhibitor with all eight liver samples. The apparent K_i values for the different human livers are listed in Table 2. With the exception of sample 7, the K_i values are in the same order of magnitude (mean 0.23 mM for all eight samples).

This study shows that furosemide, commonly used together with azathioprine after kidney transplantation, can inhibit competitively the conversion of azathioprine to 6-mercaptopurine in human liver *in vitro*. If this interaction also takes place *in vivo*, diminished immunosuppressive effect may occur since this biotransformation is necessary for the activity of the drug. From the present study it cannot be decided if the interaction is clinically important since some extrahepatic metabolism also occurs. Concentrations of furosemide used in our study can, however, be found in patient plasma samples (0.15 mM) after high doses, especially if kidney function is impaired [10] as after transplantation. Thus our results necessitate clinical study *in vivo* to determine whether furosemide inhibits the immunosuppressive effect of azathioprine in this setting.

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Effect of insulin and oral antidiabetics on glucose appearance and disappearance in the blood of rabbits

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Hypoglycemia due to insulin administration is caused by inhibition of glucose appearance [1, 2], stimulation of glucose disappearance [3, 4] or by a combination of both factors [2, 5–8]. The decrease in glucose entry into the circulating blood is the consequence of a direct action upon the liver metabolism, i.e. inhibition of glycogenolysis and gluconeogenesis, both of which result in reduction of hepatic glucose output [8–11]. The increase in glucose removal is caused by the enhancement of hepatic and peripheral glucose uptake and utilization [8, 12, 13].

Since the blood glucose lowering properties of sulfonylureas originate mainly from insulin release from pancreatic β-cells, it appeared to be of interest to study the influence of both endogenous insulin—which is released by sulfonylureas—and exogenous insulin—which has been administered—upon the kinetics of glucose.

In earlier studies, only the effect of D 860 was investigated in this respect. The authors came to the conclusion that D 860 inhibits glucose entry into the blood rather than showing an effect on removal from the blood [14–20]. However, in other studies a stimulation of peripheral glucose utilization is suggested [21, 22].

Besides two well known sulfonylureas, one belonging to the first (D 860)* and the other to the second (HB 419)† generation, two new oral antidiabetics, namely HB 699 [23]‡ and HB 180 [24]§, have been investigated in regard to their influence upon glucose kinetics.

Groups of 6–8 domestic rabbits of mixed breed, each weighing between 2.5 and 3 kg, were used in the present investigations. Prior to the beginning of the study they were subjected to a 20 hr fasting period.

The experimental animals received 35 μCi/kg D-[U-C^{14}]-glucose (Amersham CFB 96; 230 mCi/mM) intravenously. Immediately after the injection of this tracer bolus, the intragastral administration of oral antidiabetics or the subcutaneous injection of insulin in the dose indicated took

* *N*-(*p*-tolysulfonyl)-*N'*-butylurea.

† *N*-4-[2-(5-chloro-2-methoxybenzamido)-ethyl]-phenylsulfonyl-*N''*-cyclohexyl-urea.

‡ 4-[2-(5-chloro-2-methoxybenzamido)-ethyl]-benzoic-acid.

§ *N*-4-[2-(*N'*-methyl-*N'*-pyridylureido)-ethyl]-phenylsulfonyl-*N''*-4-methyl-cyclohexyl-urea.