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.

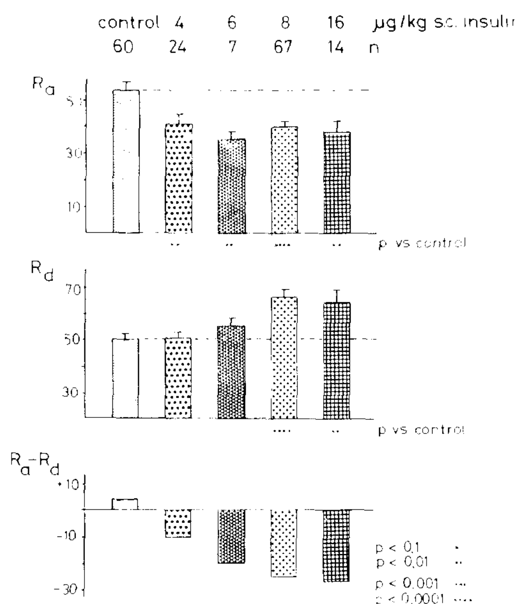

Fig. 1. Dose dependancy of insulin on glucose kinetics (15–75 min) in fasting rabbits. R_a, glucose appearance (15–75 min); R_d, glucose disappearance (15–75 min);

$$R = \frac{\text{mg glucose}}{100\ \text{ml} \times \text{hr}};\ n = \text{number of animals.}$$

place. The insulin preparations used were of porcine origin and, subsequent to a threefold crystallization, they were chromatographed on a molecular sieve. The insulin dose amounted to 16 [0.4 i.u.], 8 [0.2 i.u.], 6 [0.15 i.u.] and 4 [0.1 i.u.] µg/kg. Four different compounds were subjected to investigation in the following doses: D 860: 50 and 500 mg/kg; HB 419: 0.02 and 0.2 mg/kg; HB 699: 50 mg/kg; HB 180: 0.5 mg/kg.

Blood samples (0.1 ml) were withdrawn from auricular veins at periodic intervals and pipetted into 0.1 ml of a solution containing 0.1% heparin. Afterwards the samples were diluted immediately with digitonin/maleinimid (each 0.01%) and stored at + 4°. Subsequent determination of glucose was performed by using the hexokinase method [25]. Another blood sample was deproteinized with trichloroacetic acid [1, 7]. After neutralization, free glycerol was converted to α-glycerophosphate by incubating the samples with glycerokinase (Boehringer, Mannhein) and ATP (Boehringer, Mannheim). The determination of radiochemically pure ^{14}C-glucose required its careful separation from all radioactive metabolites. This task was accomplished by employing a mixed-bed ion-exchange resin, namely amberlite MB 3 [26], which was purchased from Serva, Heidelberg. Measurements of the resin eluates confirmed that there was no loss in glucose content during mixed-bed resin procedure. Radioactivity was determined by liquid scintillation counting.

In order to ascertain a complete elimination of ^{14}C-glucose metabolites, the samples were examined by thin-layer chromatography and scanned prior to and following purification. In none of the cases could any metabolite of ^{14}C-glucose be detected after purification. Taking blood glucose values on one hand and specific radioactivity on the other, the rates of glucose appearance (R_a) and of glucose disappearance (R_d) were calculated [5].

When the blood sugar levels are changing,

$$R_a = \frac{(B_0 - B_t)\log i_0/i_t}{\Delta t \cdot (\log B_0/B_t)}$$

and $R_d = (B_0 - B_t) + R_a$, where B_0 is the initial glucose concentration, B_t is the glucose concentration at time t, and i_0 and i_t are the initial and final specific activities [5].

The equation applied [5] was based upon administration of a single tracer dose under non-steady-state conditions [27]. The linear time-dependent decline of specific radioactivity of glucose logarithmically plotted was ascertained in each experiment and constituted a prerequisite for the application of this equation. The validity of the bolus method in comparison to the infusion technique was confirmed [28, 29].

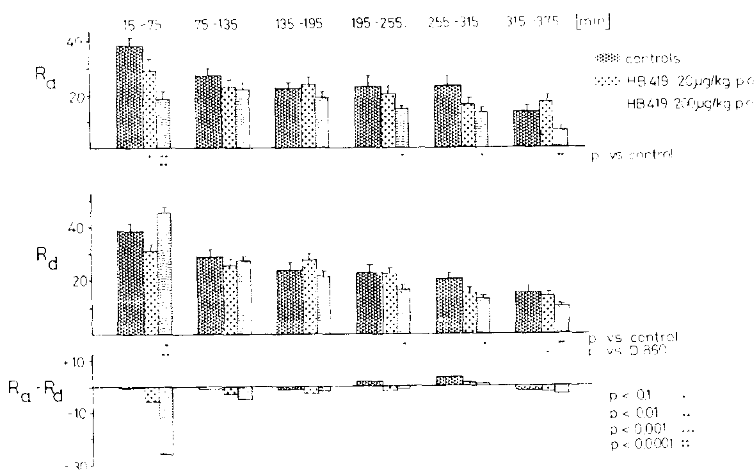

Fig. 2. Longterm effect of HB 419 (20 and 200 µg/kg p.o.) on glucose appearance (R_a) and glucose disappearance (R_d), and R_a–R_d in the blood of fasting rabbits.

$$R = \frac{\text{mg glucose}}{100\ \text{ml} \times \text{hr}}.$$

Number of animals n = 20 (0.2 mg/kg), 14 (0.2 mg/kg).

Table 1. Effect of oral antidiabetics on glucose kinetics in the blood of fasting rabbits*

	Dose	Number of animals		15–75 min R_a	15–75 min R_d	75–150 min R_a	75–150 min R_d
Control	—	22		47.1	41.3	34.3	41.0
			$s_{\bar{x}}$	5.3	2.9	3.4	2.5
D 860	50 mg/kg	16		29.2	43.4	24.2	34.4
	p.o.		$s_{\bar{x}}$	3.5	2.8	2.5	2.2
			$P_1<$	0.02	0.5	0.05	0.05
	500 mg/kg	12		25.9	49.2	22.6	28.0
	p.o.		$s_{\bar{x}}$	3.6	3.4	2.2	2.4
			$P_1<$	0.01	0.1	0.05	0.002
			$P_2<$	0.5	0.2	0.5	0.1
HB 699	50 mg/kg	14		31.9	40.7	25.9	37.9
	p.o.		$s_{\bar{x}}$	5.5	4.1	3.4	3.3
			$P_1<$	0.05	0.5	0.1	0.5
			$P_2<$	0.5	0.5	0.5	0.2
HB 419	0.02 mg/kg	14		29.4	35.6	22.9†	25.5†
	p.o.		$s_{\bar{x}}$	4.2	3.2	3.5	3.4
			$P_1<$	0.1	0.5	0.5	0.5
			$P_2<$	0.05	0.5	0.5	0.5
	0.2 mg/kg	18		30.0	51.7	25.7	39.6
	p.o.		$s_{\bar{x}}$	3.7	3.4	2.7	2.7
			$P_1<$	0.02	0.05	0.05	0.5
			$P_2<$	0.5	0.05	0.5	0.2
HB 180	0.5 mg/kg	15		24.8	51.1	28.9	38.6
	p.o.		$s_{\bar{x}}$	2.7	3.2	2.5	2.8
			$P_1<$	0.005	0.05	0.2	0.5
			$P_2<$	0.2	0.05	0.5	0.5

* Rates of glucose appearance (R_a) and of glucose disappearance (R_d) in the blood sugar lowering phase (15–75 min) and in a subsequent period (75–150 min).

$$R = \frac{\text{mg glucose}}{100\ \text{ml} \times \text{hr}}$$

P_1: t-test vs control. P_2: t-test vs D 860 group (50 mg/kg). $s_{\bar{x}}$: standard deviation.

† Calculated from 75–135 min.

For the purpose of studying the recycling phenomenon, 2-^{3}H-Glucose (Amersham TRK 361) was employed in separate trials. Identical methods for sample preparing were applied, with the exception that a two fold lyophilization was necessary to eliminate all tritium water.

The experiments were performed over a period of 150 min and the results are expressed as rates of glucose appearance (R_a) and glucose disappearance (R_d). Following the administration of insulin, after the initial hypoglycemic phase, the blood glucose levels start to rise again after 75 min. After the administration of oral antidiabetics, the blood sugar remains at the low level. The rates were therefore calculated for two separate intervals, namely the blood glucose lowering phase (15–75 min) and the counter regulation phase (75–150 min).

Subcutaneous administration of 16, 8 or 6 μg/kg insulin induced a R_d stimulation in the first phase: however, with 4 μg/kg this effect could not be observed (Fig. 1). The second phase (75–150 min) was characterized by a slow rise of blood sugar levels deriving from both increase of R_a and decrease of R_d rates. The results of experiments with equipotent oral doses [19] of the four oral antidiabetics reflecting the blood glucose lowering action are shown in Table 1.

Following the application of D 860 (50 mg/kg) or HB 699 (50 mg/kg) within the first phase (15–75 min), only a decrease of R_a is observed. With the highly active compounds HB 419 (0.2 mg/kg) or HB 180 (0.5 mg/kg), an additional stimulation of R_d can be recognized.

In the second phase (75–150 min) of the experiment, all four oral preparations caused a persistence of decreased glucose entry rates. For HB 419 and HB 180, however, the rates of glucose removal had returned to normal in the second phase.

A reduced dose of HB 419 (0.2 mg/kg) does not influence R_d at all, which is in contrast to the larger dose of 0.2 mg/kg HB 419 (Table 1). On the other hand, by raising the amount of D 860 to 500 mg/kg, an increase of R_d becomes evident (Table 1). The long-term experiments lasting over a period of 6 hr revealed that HB 419 is a typical example of a two-phase action. Administration of 0.2 mg/kg of this antidiabetic agent caused an increase in glucose disappearance during phase I. But inhibition of glucose appearance could be observed over the entire 6 hr period with HB 419 (Fig. 2) as well as with D 860.

In order to avoid our results being impaired by partial recycling [31] of ^{14}C-glucose metabolites, the experiments were repeated by using 2-^{3}H-glucose [32], since in this case recycling is not possible. It could be demonstrated that all the effects observed with U-^{14}C-glucose are in agreement with the results obtained with 2-^{3}H-glucose.

The findings of our studies in which the effects of exogen-

ous administered insulin were compared with those of the endogenous released hormone are in accordance with previously published results [1, 2, 5–9, 19, 20]. A decline of blood glucose levels following the administration of oral antidiabetics in standard doses is caused mainly by inhibition of liver glucose output, while blood glucose decrease by exogenous insulin (8 μg/kg) is due to inhibition of glucose output as well as to stimulation of glucose uptake. The experiments with various doses or oral antidiabetics emphasize that smaller doses (0.02 mg/kg HB 419 or 50 mg/kg D 860) only cause an R_a inhibition, while larger doses (0.2 mg/kg HB 419 or 500 mg/kg D 860) additionally cause a stimulation of R_d (Table 1). Therefore, peripheral effects of endogenous insulin can only be accomplished if the secreted insulin exceeds a certain amount, high enough to pass the liver.

This explanation is in agreement with experiments [33] which have shown a significant rise of insulin in the portal vein as early as 10 min after HB 419 administration, while a rise in peripheral insulin can only be observed after 45 min. In addition, there is no difference in peripheral immunoreactive insulin after administration of equipotent doses of HB 419 or D 860 [34]. The above concept is supported by similar dose-dependent effects of exogenous insulin on R_d (Fig. 1). These results are of clinical relevance, since Sönksen [2] reported similar findings in connection with the infusion of varying doses of insulin in man.

Following the administration of exogenous insulin, the hypoglycemic phase (15–75 min) is characterized by inhibition of R_a and stimulation of R_d. During the subsequent rise in blood sugar levels (75–150 min), a reversal of the previously observed glucose turnover rates can be recognized [7]. This is a good example for the coordinated antagonism in liver carbohydrate metabolism in regard to relative excess or deficiency of insulin [35].

The stimulation of R_d by insulin or oral antidiabetics ends after the blood glucose lowering phase (15–75 min). In regard to inhibition of R_a, the actions of oral antidiabetics are characterized by a long-term inhibition of liver glucose output, while exogenous insulin has only a short-time effect. This long-term inhibition may be explained by long-term insulin release. Other possibilities may be either the amplification of insulin action upon the liver, or influence on counter-regulatory hormones [36,37], or a long-term release of the drug from a pool [38].

In summary, it can be stated that low doses of exogenously administered insulin as well as endogenously released insulin by low doses of oral antidiabetics result in a decrease of blood glucose levels due to inhibition of glucose appearance. In contrast to this, treatment with large doses of insulin or oral antidiabetics not only inhibit glucose entry into the circulating blood but they also cause enhancement of glucose removal from the blood.

The results of experiments performed over a prolonged period of time show that the rather long lasting phase of blood glucose decrease after HB 419 or D 860 is mainly caused by inhibition of glucose appearance.

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