Furthermore, to ascertain that the small size of the nigral sample would not distort the results of the assay, nigrae were pooled from four mice and processed for the assay. This experiment provided an MDA nigral value of 1.3 nmol/mg protein.

Others have reported regional levels of MDA in the rat brain. Mizuno and Ohta [9] found low MDA levels in the nigra, but their animals were killed under barbiturate anesthesia, which both reduces the level of peroxidation and interferes with the TBA assay. MDA values reported by Noda *et al.* [10] were similar to ours in other regions, but not in the SN. Species differences, long time after sacrifice, technique of sampling, and absence of neuromelamin in rats [11] may all contribute in various degrees to this discrepancy.

Our findings may have implications relevant to the theory of aging and neuronal degeneration. The septal area is a predominantly cholinergic structure and cholinergic neurons are primarily affected in primary degenerative dementia of the Alzheimer's type [12]. SN neurons are depleted during physiological aging [1] and even more so in all neurodegenerative processes associated with Parkinsonian symptoms [13]. The high content of MDA may predipose these areas to damaging effects of the pathogenic noxae.

The SN contains the highest levels of MDA and iron [14], the lowest levels of the scavenger GSH [15] and ubiquinone Q10 with a skewed ratio between the reduced and oxidized form in favour of the oxidized Q10 [16]. All these factors suggest that this mesencephalic nucleus may be particularly vulnerable to oxidative stress and has an intrinsically reduced capability to cope with it.

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Purification and partial characterization of human intestinal glutathione *S*-transferases

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Glutathione *S*-transferases (EC 2.5.1.18) are a family of multifunctional enzymes which play an important role in detoxification, or storage and transport, of xenobiotics and physiologic compounds, in a wide variety of tissues and species [1–4].

In humans, the enzyme system is present in most, if not all epithelial tissues [2, 3], but is most studied so far in the liver [2, 3, 5–11]. However, the intestinal tract is the first route of many toxic compounds, digested with food or drugs. The intestine, in particular the epithelial mucosal cells, are primarily exposed to dietary xenobiotics, and a proper functioning of the detoxificating enzyme system, like the glutathione *S*-transferases, is very important here. Little is known about human intestinal glutathione *S*-transferases [12–14]. In this study adult human intestinal glutathione *S*-transferases are purified and partially characterized. Their properties are compared with those of human liver enzymes.

Materials and methods

Tissue. Human tissue was obtained after autopsy or surgical resections. Liver tissue was obtained from a kidney donor who died by cerebral damage after a traffic accident. Two specimens, one of jejunum and one of ileum, were obtained from another kidney donor, who also died by cerebral damage. Other ileum specimens were obtained

from a patient with a coecal tumor, and from a patient suffering from Crohn's disease. All tissue used was macroscopically normal.

Tissue was washed in ice-cold 0.9% NaCl solution, as soon as possible, and was stored at −80° or used immediately.

Purification of glutathione S-transferases. Liver was cut in pieces and homogenized in a Waring Blendor for 20–30 sec in 20 mM Tris/HCl buffer pH 7.4, containing 0.25 M sucrose. Further homogenization was performed by 5 strokes at 1000 rpm in a glass–Teflon homogenizer.

Intestinal mucosal cells were scraped off carefully with a scalpel, and the cells were homogenized by 5 strokes at 1000 rpm in a glass–Teflon homogenizer, in the medium described above. All handling was done on ice.

The 150,000 *g* supernatant of both tissues was used for isolation of total glutathione *S*-transferases. This was done by affinity chromatography on glutathione-agarose (Sigma Chemicals, St. Louis, MO) essentially as described by Singh *et al.* [5]. The only modification was the exchange of 2-mercapto-ethanol by the less toxic dithiothreitol (Sigma Chemicals).

Miscellaneous. Glutathione *S*-transferase assay, with 1-chloro 2,4-dinitrobenzene (Sigma Chemicals) as substrate, was performed on the GSH-agarose purified enzyme, as described by Habig *et al.* [15]. SDS polyacrylamide gel electrophoresis (10% w/v acrylamide) was done as described before [16] and isoelectric focusing on polyacrylamide gels, with Ampholines of pH range 3.5–10 (LKB, Bromma, Sweden) was done by the method of O'Farrell [17]. The pH range was estimated as follows: the gel was cut in pieces of 0.4 cm, then 1.0 ml of distilled water was added and after mild shaking for about 16 hr the pH was measured. Protein was determined by the method of Lowry [18].

Results and discussion

Figure 1 shows the purified glutathione *S*-transferases (GSH transferases) from human intestine and liver, after SDS polyacrylamide gel electrophoresis. The preparation from liver is composed of subunits with a molecular mass of 25,000 Da (slot 2) whereas the intestinal preparations contain subunits with molecular masses of 25,000 and 24,000 Da (slots 3 and 4).

Human liver GSH transferases predominantly are composed of 25,000–27,000 Da subunits which correspond mainly with basic forms of the enzyme [6–9]. Less intense bands with slightly higher molecular mass are seen in some preparations [6, 7, 9]. The latter subunits belong to the so-called "near neutral" forms of the enzyme. Generally the acidic forms of the enzyme are composed of subunits with the lowest molecular mass and represent only a few percent of the total human liver GSH transferases [6, 7, 10].

The 25,000 Da band in the liver preparation shown in Fig. 1 also corresponds mainly with basic GSH transferases as can be seen in Fig. 2. Figure 2 shows the 500 nm scans of the protein pattern of hepatic (panel A) and of intestinal GSH transferases (panels B and C), after isoelectric focusing in polyacrylamide gels. The liver preparation contains mainly basic and "near neutral" forms (76 and 17% of total, respectively) while the intestinal preparations are composed mainly of basic (60%) and acidic forms (25%). Thus the bands of lowest molecular mass seen in Fig. 1 in the intestinal preparations (slots 3 and 4) may correspond to the acidic form(s) of the enzyme, which are nearly absent in the liver preparation (see Fig. 2, peaks 4 and 5). Recently GSH transferases were purified from human skin and here also acidic forms have the lowest subunit molecular mass [19]. Several other differences between hepatic and intestinal GSH transferase composition may be present. The basic form, denoted peak 2 in Fig. 2, panels B and C, may be absent in the liver preparation (Fig. 2, panel A). On the other hand in the "near neutral" area of the liver preparation several proteins are present which are absent in both intestinal preparations. A difference between both intestinal preparations may be the absence of a basic form of GSH transferase (Fig. 2, panel B, peak 1) in one ileal preparation (panel C).

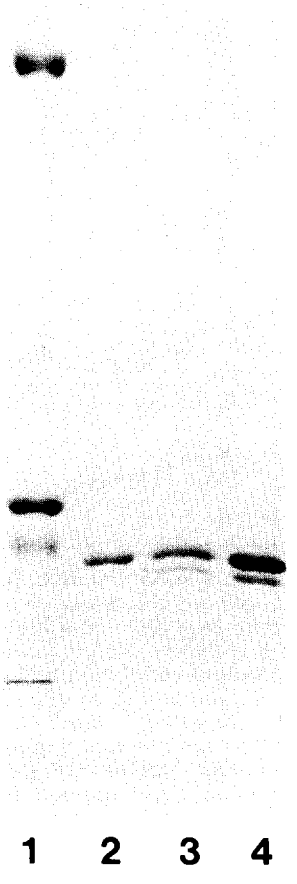

Fig. 1. SDS polyacrylamide gel electrophoresis of purified GSH transferases from human intestine and liver. Slot 1 contains markers proteins with molecular masses of 68,000 (upper band), 29,000 and 24,000 Da respectively. The dye front is indicated by the narrow band in the lower part of slot 1. Purified GSH transferases are shown in slot 2 (liver, 3 μg protein) slot 3(ileum, 3.2 μg protein) and slot 4 (ileum, 4.5 μg protein). The ileal preparation in slot 3 originates from the patient with coecal tumor (see Materials and methods). Molecular masses of GSH transferase subunits are approximately 25,000 (upper band) and 24,000 Da for intestinal, and 25,000 Da, for hepatic preparations.

However, the main difference between liver and intestinal preparations seems to be the high content of acidic GSH transferases in the intestinal mucosal cells. High contents of acidic GSH transferases have been reported in human erythrocytes [6, 20], lung [20], heart [20], breast

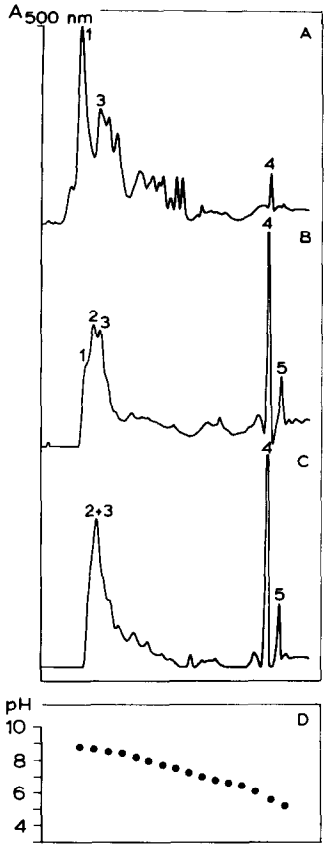

Fig. 2. 500 nM scans of purified GSH transferases, from human intestine and liver, after isoelectric focusing. Purified GSH transferases were subjected to isoelectric focusing on polyacrylamide rod gels with a pH gradient of 3.5–10. After isoelectric focusing and staining, gels were scanned at 500 nM. Preparations are identical to that shown in Fig. 1 and Table 1. In panels A, B and C, purified GSH transferase preparations from liver (96 μg protein), ileum (80 μg protein) and from another ileum specimen (64 μg protein) are shown respectively. Numbers are explained in the text. Panel D shows the pH gradient in the gels.

[21] and placenta [6]. For adult human intestine no data on GSH transferase composition are available until now. In fetal human intestine, like in fetal lung, kidney, spleen and brain tissue, only acidic forms of GSH transferases are present [12, 22, 23].

The specific activities of GSH-agarose purified intestinal and hepatic GSH transferases, determined with 1-chloro 2,4-dinitrobenzene as a substrate, are comparable. Values for hepatic and intestinal GSH transferases, which are shown in Table 1, are 32.0 and 27.9–38.1 μmol/mg protein · min, respectively. GSH transferases, isolated from ileal tissue of a patient with a coecal tumor had much lower specific activity (16.7 μmol/mg protein · min). This may be well explained by the great interindividual variations in GSH transferase activities, as determined in 9000 *g* fractions of intestinal tissue [14]. Another explanation may be that of a low content of GSH transferase activity is coupled to a higher risk of getting carcinomas. This will be investigated further.

The value of 32.0 ± 1.3 μmol/mg protein · min for the GSH-agarose purified total GSH transferase population from human liver, corresponds very well with earlier published values of 17.9–65.1 μmol/mg protein · min for ten different adult liver preparations [6–8].

Table 1. Specific activities of purified GSH transferases from human intestine and liver

Tissue	Specific activity (μmol/mg protein · min)	
Liver*	32.0 ± 1.3	N = 6
Jejunum†	33.5 ± 3.4	N = 3
Ileum†	27.9 ± 1.2	N = 3
Ileum‡	16.7 ± 0.5	N = 3
Ileum*	38.1 ± 2.0	N = 3

Specific activity from GSH-agarose purified preparations was determined with 1-chloro 2,4-dinitrobenzene as substrate, and is expressed with SD for the given number of determinations (N).

* These preparations are also shown in Figs 1 and 2.

† Tissue from the same patient.

‡ Tissue from the patient with coecal tumor (see Materials and methods).

The main conclusions from this study are: (1) human adult intestinal GSH transferases are in part similar to those of the liver; however, some acidic forms are present which are nearly absent in liver; (2) the intestinal GSH transferases are composed of more low molecular mass subunits (24,000 Da), as compared to the liver enzymes; (3) the specific activity of intestinal GSH transferases is comparable to that of hepatic enzymes. This indicates the importance of the intestinal mucosa in the protection of the body against toxic compounds, present in food, drugs and other intakes.

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Interaction of civetone with rat liver microsomal cytochrome P-450 and steroidogenic enzymes

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Civetone (9-cycloheptadecen-1-one) is a component of the animal secretion, civet. The characteristic musky odour of civetone becomes pleasant at very dilute concentrations, hence its use in the perfumery industry. This 17-membered macrocyclic ketone contains one ethylenic double bond, therefore it can exist as either the *cis* or *trans* isomer. Naturally occurring civetone has been identified as the *cis* isomer and bears some structural resemblance to a steroid [1]. The biological properties of civetone have not been characterised.

Another naturally occurring macrocyclic ketone is muscone (3-methylcyclopentadecanone), which is responsible for the odour of musk. Unlike civetone, there is some data about the biological properties of muscone. Pretreatment of rats with muscone causes induction of rat liver microsomal cytochrome P-450. In addition, a Type I binding spectrum was produced on addition of muscone to microsomes [2]. In this study, we report the interaction of civetone with rat liver microsomal cytochrome P-450 and assess its inhibitory activity *in vitro* against the aromatase, cholesterol side chain cleavage (CSCC) and steroid 5 α-reductase enzymes. Inhibitors of such steroidogenic enzymes may provide leads in the development of useful therapeutic agents for the treatment of hormone dependent breast and prostate tumours.

Materials and methods

Chemicals. NADP, glucose-6-phosphate, glucose-6-phosphate dehydrogenase and NADPH were obtained from Boehringer (Mannheim, F.R.G.). All non-radioactive steroids were purchased from Sigma Chemical Co. (St Louis, MO). [26-^{14}C]Cholesterol (specific activity 56 mCi/mmol) and [1,2-^{3}H]androstenedione (specific activity 48 Ci/mmol) were from New England Nuclear (Boston, MA). [4-^{14}C]Testosterone (specific activity 50–60 mCi/mmol) was from Amersham International (U.K.). Aminoglutethimide was a gift from Ciba Geigy Ltd. (Horsham, U.K.). Samples of optically pure *cis* and *trans* iso-