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1-NAPHTHOL CONJUGATION IN ISOLATED CELLS FROM LIVER, JEJUNUM, ILEUM, COLON AND KIDNEY OF THE GUINEA PIG

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Abstract—Cells from liver, jejunum, ileum, colon and kidney of the guinea pig were freshly prepared by standard isolation procedures. Cells were incubated in the presence of several concentrations of 1(^{14}C)-naphthol, and formation of 1-naphthol glucuronide and 1-naphthol sulphate was assessed at various times by thin layer chromatography. Cells from all five tissues conjugated 1-naphthol. The velocity of conjugation was fastest in jejunals cells (2.9 nmole/mg prot × min) followed by cells from liver, ileum, colon and kidney (0.2 nmole/mg prot × min). The apparent K_m-values for glucuronidation ranged from 17 μM to 32 μM, and those for sulphation from 15 μM to 35 μM. Each tissue had a specific conjugation pattern. The two extremes were kidney cells, which had a glucuronidation/sulphation ratio in excess of 10, and colon cells which had a ratio of 0.38. The data suggest that these tissues possess different levels of 1-naphthol-conjugating enzymes, which resemble with regard to their apparent substrate affinities.

There is now increasing interest in the role which extrahepatic organs such as intestine (cf. [1]) and kidney (cf. [2]) have in glucuronidation and sulphation of drugs. The contribution of each organ cannot be simply estimated on the basis of available data, because the experimental designs of different laboratories usually differ with regard to species, substrates, or modes of evaluation. To get around this difficulty, some recent studies have directly compared the drug metabolizing activities of liver with those of other organs, using microsomes [3] or isolated cells [4–6]. Yet a major question is still unsolved: Do the glucuronyltransferases and sulphotransferases of different tissues exhibit similar substrate affinities, and at which levels are the enzymes present in each tissue? To approach the answer, we studied conjugation of 1-naphthol in isolated cell preparations from 5 major drug-metabolizing tissues under identical conditions.

1-Naphthol was chosen as substrate, because it is readily conjugated with sulphate and glucuronic acid in cell preparations from liver [7, 8] intestine [5, 9, 10], and kidney [6], or tissue cultures of colon [11]. In addition 1-naphthol is rapidly taken up by cells [7], and transport is not a limiting step in the conjugation reaction. As experimental species we used the guinea pig, because its intestinal cells are easier to handle than those of rat [12, 13], and cells from intestine [12] and liver [14, 15] have recently qualified as valuable in drug metabolism studies.

MATERIALS AND METHODS

Chemicals. 1(1^{14}C)-Naphthol (56 mCi/mmole) was obtained from Radiochemical Centre (Amersham, England). Collagenase was from Boehringer (Mannheim, F.G.R.), minimum essential medium with Earl's salts (MEM) was from Serva (Heidelberg, F.R.G.). Silica gel G thin layer foils were from Merck (Darmstadt, F.R.G.). All other chemicals were obtained at the highest purity from Merck.

Animals. Male guinea pigs (300–400 g) were obtained from Ivanovas (Kisslegg, F.R.G.). The animals were fed a standard guinea pig chow and water *ad lib.*

Cell isolation. Liver cells were isolated according to Hauber and colleagues [15] by perfusing the liver first with Ca^{2+}-free, oxygenated Hank's medium for 6 min and then with MEM, supplemented with 20 mM Hepes-buffer containing 0.1% collagenase (15 min). Jejunal epithelial cells were obtained from the first quarter of the small intestine, ileal cells from the last quarter, and colon cells from the whole colon between coecum and rectum. These intestinal epithelial cells were isolated according to the method of Hegazy *et al.* [13], by treatment of the intestinal lumen with media, containing citrate, EDTA and dithiotreitol. Kidney tubule fragments were prepared following the method of Guder and co-workers [16]: The kidneys were briefly perfused and excised. The marrow was removed. Each cortex was cut in 5 slices. These were then passed through a nylon sieve (pore size 0.8 × 0.8 mm). The tissue mash was treated with collagenase-containing MEM for 45 min. Cell preparations from all organs were filtered (pore size 0.25 mm × 0.25 mm), washed twice by centrifugation (60 *g*, 2 min) in MEM, and stored in MEM at 0°.

Cell viability. Each preparation was tested with regard to its microscopic morphology, and respiration. All preparations had oxygen consumption rates in excess of 10 nmole/mg prot × min and were inhibited by oligomycin more than 50% (cf. [13]).

Standard incubation. Cells (1.7–1.9 mg protein) were incubated in 1.5 ml of oxygenated MEM (containing 0.1 mM cystin and 1 mM sulfate), sup-

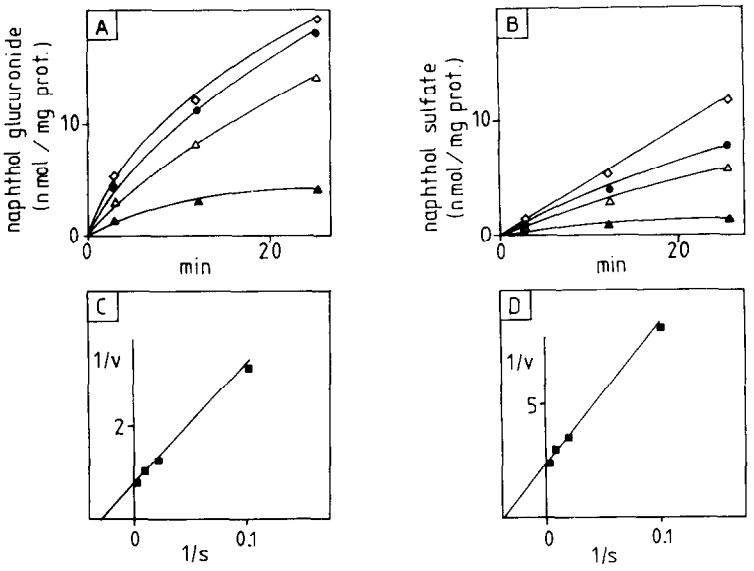

Fig. 1. 1-Naphthol conjugation in liver cells. Standard incubation of liver cells. ^{14}C-Naphthol was added at the following concentrations: 10 μM (▲); 50 μM (△); 125 μM (●); 300 μM (◇): (A) time course of naphthol glucuronide formation; (B) time course of naphthol sulfphate formation; (c) Lineweaver–Burk plot of naphthol glucuronide; (D) Lineweaver–Burk plot of naphthol sulphate. v is expressed as nmole conjugate/mg cell protein × min, and s is expressed in μM.

plemented with 20 mM Hepes (pH 7.3) at 37° while stirring. After 15 min of preincubation 1(^{14}C)-naphthol (1.5 μCi) was added at various concentrations. The optimal range of concentrations has been determined for each cell type in preliminary experiments. Samples of 200 μl were withdrawn after 15 sec and at various times thereafter, and transfered to 400 μl of a mixture of methanol and chloroform (1:3). After shaking for 30 sec, another 600 μl of chloroform were added.

Analysis of 1-naphthol conjugates. 50 μl of the aqueous phases were applied to silica gel thin layer chromatography foils and 1-naphthol glucuronide was separated from 1-naphthol sulphate in a solvent system, consisting of *n*-butanol:0.01 M Tris:propionic acid (75:14:1.1). The r.f. values were 0.26 for 1-naphthol glucuronide and 0.69 for 1-naphthol sulphate [7]. The foils were cut in 20 fractions. Each fraction was placed in a vial for liquid scintillation counting.

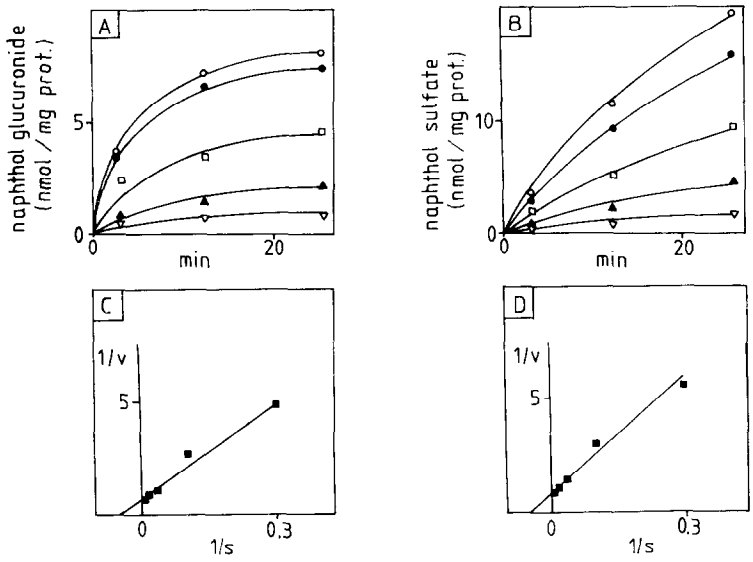

Fig. 2. 1-Naphthol conjugation in jejunal cells. Standard incubation of jejunal cells. ^{14}C-Naphthol was added at the following concentrations: 3.5 μM (▽); 10 μM (▲); 30 μM (□); 60 μM (●); 100 μM (○). Panels and units as in Fig. 1.

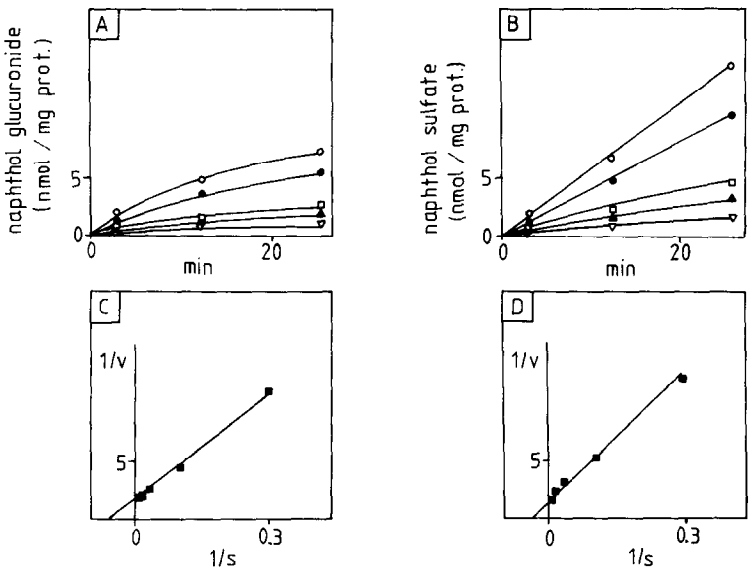

Fig. 3. 1-Naphthol conjugation in ileal cells. Standard incubation of ileal cells. ^{14}C-Naphthol was added at the following concentrations: 3.5 μM (▽); 10 μM (▲); 30 μM (□); 60 μM (●); 100 μM (○). Panel and units as in Fig. 1.

Cellular protein was determined with the Biuret method. All experiments were performed in duplicates with 5 different cell preparations. The depicted time courses of conjugation and the corresponding Lineweaver–Burk plots show typical results (duplicate determinations) from single preparations. Table 1 shows the mean values from 5 preparations.

RESULTS

The upper panels of Figs. 1–5 show the time courses of glucuronidation and sulphation in the 5 cell types. Initial rates of uptake were estimated graphically from the slopes after 3 min of incubation. The initial rates were plotted according to Lineweaver–Burk (lower panels). In all cell types, except colon cells (Fig. 4) the rate of glucuronidation exceeded the rate of sulphation. Kidney tubule fragments sulphated very poorly (Fig. 5), not allowing kinetic evaluation in a Lineweaver–Burk diagram. In cells from liver, jejunum and ileum the glucuronide/sulphate ration shifted in favour of the sulphate conjugate with increasing length of incubation. The sulphation rates in jejunal and ileal cells were higher

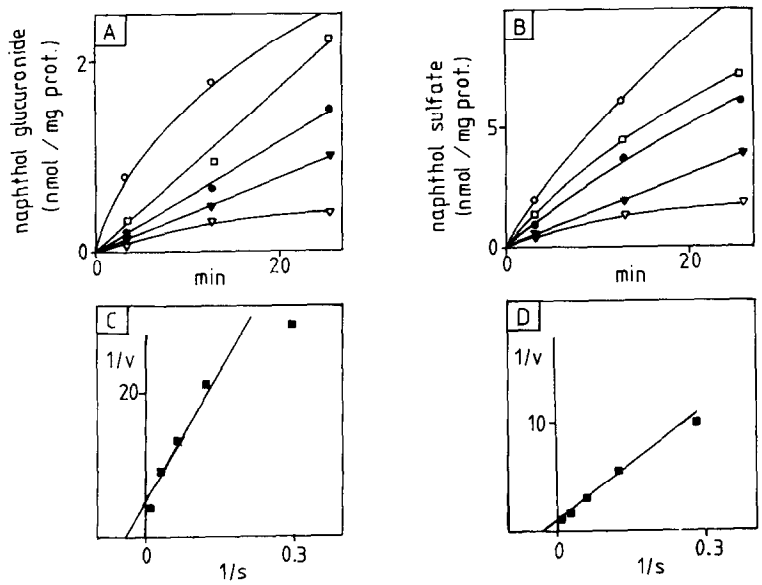

Fig. 4. 1-Naphthol conjugation in colon cells. Standard incubation of colon cells. ^{14}C-Naphthol was added at the following concentrations: 3.5 μM (▽); 8 μM (▼); 16 μM (●); 33 μM (□); 100 μM (○). Panels and units as in Fig. 1.

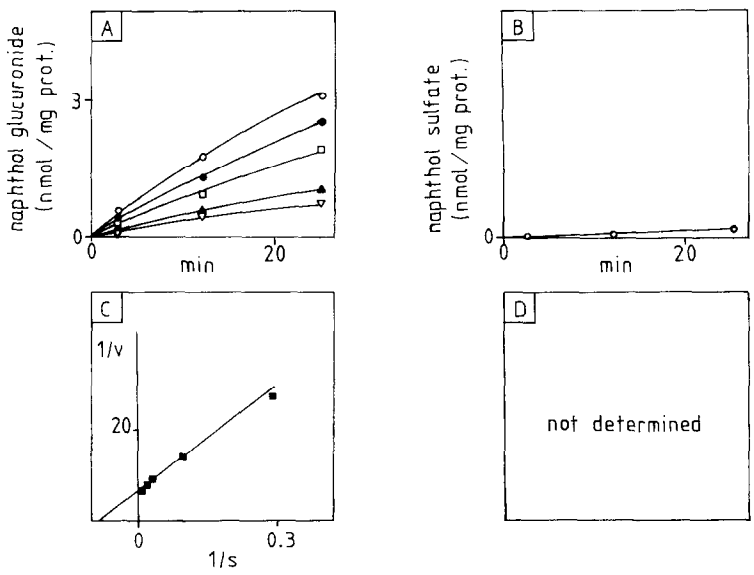

Fig. 5. 1-Naphthol conjugation in kidney cells. Standard incubation of kidney tubule fragments. ^{14}C-Naphthol was added at the following concentrations: 3.5 μM (▽); 10 μM (▲); 30 μM (□); 60 μM (●); 100 μM (○). Panels and units as in Fig. 1.

than in a previous study [17]. This difference is due to the use of MEM as incubation medium, instead of a sulphur-depleted modified medium previously used.

The results from the Lineweaver–Burk plots are summarized in Table 1. Jejunal cells had the highest overall conjugating capacity. Ileal cells and liver cells ranged second place, followed by colon cells. Kidney cells conjugated 1-naphthol poorly. The K_m-values of glucuronidation ranged from 17 μM to 32 μM and those of sulphation from 15 μM to 35 μM.

DISCUSSION

Isolated cells possess both, the conjugating enzymes of the cytosol and of the endoplasmic reticulum. Therefore they allow us to study simultaneously the kinetics of sulphation and glucuronidation. Whereas most previous studies on conjugation in isolated cells have employed only 1 or 2 substrate concentrations, the present investigation aimed at comparing the apparent K_m-values, the maximal velocities of each reaction, and the conjugation patterns of cells from different organs. The usefulness of the guinea pig for this study was anticipated from its high conjugating activity in the intestine [18] and further verified, because its hepatocytes (present data) conjugated 1-naphthol at a slightly higher rate than rat hepatocytes [8]. Possible limitations for a comparison between cells from different organs come from the observation of Borm *et al.* [19] that isolated cells, gained by different isolation procedures, do not completely equal with regard to their conjugating capability.

Cells from each organ exhibited a specific conjugation pattern: Kidney cells glucuronidated moderately, but sulphated very poorly, in accordance with findings in the intact organ [20]. Cells from liver and intestine, conjugated more with glucuronic acid than with sulphate like the respective organs of the rat [5, 7, 21, 22]. The rate of glucuronidation decreased from the oral part towards the aboral part of the intestine more than the rate of sulphation. In colon cells sulphation exceeded glucuronidation;

Table 1. Kinetic constants of 1-naphthol conjugation

Organ	Glucuronidation		Sulphation		Total conjugation
	K_m (μM)	V_{max} (nmole/mg prot × min)	K_m (μM)	V_{max} (nmole/mg prot × min)	ΣV_{max} (nmole/mg prot × min)
Liver cells	25.0 ± 6.0	0.8 ± 0.30	24.0 ± 5.8	0.4 ± 0.08	1.2
Jejunal cells	20.0 ± 5.0	1.7 ± 0.20	20.0 ± 3.8	1.2 ± 0.11	2.9
Ileal cells	17.0 ± 4.3	0.6 ± 0.16	15.0 ± 6.0	0.6 ± 0.09	1.2
Colon cells	32.0 ± 8.3	0.3 ± 0.09	35.0 ± 5.0	0.8 ± 0.27	1.1
Kidney cells	18.5 ± 8.0	0.2 ± 0.01	—	—	0.2

Each value is the mean from duplicate measurements in 5 cell preparations (±S.D.).
The rate of sulphation in kidney cells was too low for assessing constant.

interestingly, a similar pattern has been found in human colon [11]. Thus the tissue-specific glucuronidation/sulphation pattern seems to resemble in different species.

The glucuronide/sulphate ratio decreased in all cell types with increasing length of incubation. This is in accordance with findings on 4-hydroxybiphenyl conjugation in isolated intestinal epithelial cells [12]. A possible explanation for this shift is provided by the time courses: glucuronidation deviated early from linearity. This occurred especially at low substrate concentration. Therefore cosubstrate-depletion is probably not the cause. An alternative explanation would be the assumption that these cells have an excess of glucuronidase over sulphatase. These enzymes may also be involved in the altered 1-naphthol-conjugation pattern, in tumorous compared to normal tissue of colon [11] and lung [23].

A major aim of this study was to see whether cells from different organs contain different conjugating enzymes. The apparent K_m-values found here are in good agreement with values found by others: glucuonidation in isolated rat enterocytes was saturated at 50 μM 1-naphthol [9] suggesting an apparent K_m-value around 20 μM, and isolated rat hepatocytes exhibited an apparent K_m-value of 57 μM [8]. 1-Naphthol-sulphation in rat hepatocytes had a K_m-value of 30 μM [8] and sulphation by isolated sulphotransferase of the guinea pig exhibited a K_m-value of 25 μM [24]. Thus there are two surprising results: first the K_m-value of 1-naphthol-glucuronidation is in the same range as that for sulphation, and second the K_m-values of each enzyme in different organs are so close together that the remaining differences may be explained by experimental factors. The results are therefore compatible with the possibility that the 1-naphthol conjugating enzymes of different tissues are identical enzymes. Consequently the contribution which each organ plays in glucuronidation and sulphation of 1-naphthol is virtually independent of the 1-naphthol concentration: it may be calculated by multiplying the V_{max} values of Table 1 with the tissue mass of each organ.

A more realistic estimation of the contribution of each organ, however, must take into account, that liver, intestine and kidney are absorptive and secretory organs, performing transcellular transport. Thus the physiological contribution depends on the route of administration. Orally administered 1-naphthol undergoes a near-complete intestinal first pass conjugation [22]. Likewise free 1-naphthol in the portal vein is subject to a hepatic first pass conjugation [21] and circulating naphthol undergoes conjugation in the kidney before it is excreted in the urine [20]. If pathological conditions lead to decreased conjugation in one organ then the other organs can only partially replace its function.

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