Biochemical Pharmacology, Vol. 46, No. 8, pp. 1339–1347, 1993
Printed in Great Britain

0006-2952/93 $6.00 + 0.00

COMPARTMENTATION OF INTESTINAL DRUG SULPHOCONJUGATION

INCORPORATION OF LUMINAL AND CONTRALUMINAL [^{35}S]SULPHATE INTO 1-NAPHTHOL BY THE ISOLATED MUCOSA OF GUINEA PIG JEJUNUM AND COLON*

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(*Received* 18 *May* 1992; *accepted* 12 *July* 1993)

Abstract—Compartmentation of 1-naphthol metabolism was inferred from the metabolite pattern and distribution in the isolated mucosa of guinea pig intestine mounted in a flux chamber (Sund and Lauterbach, *Arch Pharmacol Toxicol* **58**: 74–83, 1986). To verify the existence of these compartments the dependence of [^{35}S]sulphate incorporation into 1-naphthol sulphate on the side of administration of 1-naphthol and [^{35}S]sulphate was determined. Isolated mucosae were pre-equilibrated with [^{35}S]-sulphate (5×10^6 cpm/μmol, 1 mM) for 30 min and subsequently incubated for 15 min with 50 μM 1-naphthol. The three 1-naphthol sulphate fractions (luminal side, blood side and tissue) were assayed by HPLC and liquid scintillation counting; their specific activity was calculated as percentage of the specific activity of the inorganic sulphate administered. 1-Naphthol glucuronide was also measured. In jejunal experiments: after luminal administration of 1-naphthol, 1-naphthol sulphate appeared almost exclusively in the luminal solution; its specific activity approached 70% and 3%, when [^{35}S]sulphate was added to the luminal and blood side, respectively. After introducing 1-naphthol and [^{35}S]sulphate on the blood side, a high and similar specific activity of 50–60% was observed in all three 1-naphthol sulphate fractions (luminal and blood side, tissue) though adding [^{35}S]sulphate to the lumen side decreased the specific activity to 10–20%. In experiments on colonic mucosa: a specific activity both of luminal and blood side 1-naphthol sulphate of more than 50% was observed with blood side [^{35}S]-sulphate irrespective of the side of 1-naphthol administration. When [^{35}S]sulphate was placed on the luminal side the specific activity of blood side 1-naphthol sulphate dropped to only 3%, and that of luminal 1-naphthol sulphate ranged between 6% and 20%. Supplementary experiments in which mucosae were exposed to 1-naphthol and [^{35}S]sulphate for 45 min without preincubation showed a tendency to decrease the lumen : blood distribution ratio of 1-naphthol sulphate. However, the general pattern of 1-naphthol sulphate specific activity remained unchanged. The experiments provide further evidence that the jejunal conjugation of phenolic drugs is being performed in two major compartments, which are accessible from the lumen ("luminal compartment") and blood ("systemic compartment") side. The luminal compartment seems practically inaccessible to blood side sulphate as is the systemic compartment for luminal 1-naphthol. In the colonic mucosa, a major "systemic compartment" receiving its sulphate from the blood side is the site for most of the events, but a minor "luminal compartment" seems to be involved as well.

A previous study [1] on guinea pig intestinal mucosa mounted in flux chambers [2] showed that the jejunal metabolism and metabolite transport of [^{14}C]1-naphthol varied with the side of drug administration. When these conjugates were formed after luminal administration of 1-naphthol its glucuronide and sulphate were transported almost exclusively back to the lumen. If these metabolites formed from 1-naphthol on the blood side, the distribution pattern changed entirely: the glucuronide was now transferred preferentially to the blood side, whereas the sulphate showed a more equal distribution between the lumen and blood sides. These and other findings suggested that jejunal drug metabolism and metabolite transport take place in two functionally separate compartments, accessible either from the lumen or the blood side; designated the "luminal compartment" and the "systemic compartment", respectively [3]. In the colon, however, clear-cut evidence for a similar compartmentation of phenolic metabolism was not obtained.

In a later study [4] selective omission of inorganic sulphate indicated a strong dependence of sulphoconjugation in the "luminal compartment" on luminal sulphate, whereas the "systemic compartment" seemed to utilize sulphate from both sides. Theoretically, the unphysiological absence of sulphate on one side, the resulting steep sulphate gradient in the direction lumen to blood or vice versa, and the at least partial compensatory increase in glucuronidation might have had some impact on the results. In addition, the former study was

* Dedicated to Prof. Dr Wolfgang Forth, Munich, on the occasion of his 60th birthday with thanks.

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performed at a concentration (130 μmol/L) which tended to saturate 1-naphthol sulphoconjugation at least in the colon [1] and decreased the sulphoconjugate fraction formed. Subtle differences in sulphate ion utilization from the two sides could have been obscured.

In the present study, a normal 1 mM concentration of inorganic sulphate was maintained on both sides of the intestinal mucosa whereas ^{35}S-labelled sulphate was added either to the luminal or the blood side. Thus, a normal balance between sulphoconjugation and glucuronidation was preserved under conditions which allow the external sulphate source for the postulated compartments to be identified by a determination of the extent of ^{35}S incorporation in the various sulphoconjugate fractions. 1-Naphthol at a concentration (50 μmol/L) which does not saturate colonic sulphoconjugation was administered as the unlabelled drug. At the end of incubation, the amount of 1-naphthyl sulphate (as well as of 1-naphthyl glucuronide) at the luminal side, blood side and in the tissue, and its specific radioactivity were determined.

The study also included [^{35}S]sulphate tissue uptake experiments designed to establish those conditions in which the endogenous pools of sulphate were in equilibrium with exogenously added [^{35}S]sulphate.

A preliminary account of the study has been presented [5].

MATERIALS AND METHODS

Drugs and chemicals. [^{35}S]Sodium sulphate (sp. act. 559 mCi/mmol) and [1,2-^{3}H]polyethylene glycol 900 ([^{3}H]PEG, M_r 800–1000, sp. act. 3.3 mCi/gram) were obtained from the New England Nuclear Corp. (Boston, MA, U.S.A.). 1-Naphthol of analytical grade was from E. Merck (Darmstadt, Germany). Other chemicals were of analytical or chromatographic grade.

The [^{35}S]sodium sulphate preparation was kept as a stock solution in water from which appropriate volumes were taken to dryness and dissolved in the incubation medium. Since the specific activity of the preparation was high, the experimental concentrations of radiolabel (see below) did not cause any osmolarity change in the medium.

Incubation media. The standard incubation medium had the following composition (mmol/L): NaCl 96.4, KCl 7.0, $CaCl_2$ 3.0, $MgSO_4$ 1.0, sodium phosphate buffer (pH 7.4) 0.9, Tris buffer (pH 7.4) 29.5, glucose 14.0 and mannitol 14.0. In the metabolism experiments [^{35}S]sulphate and/or unlabelled 1-naphthol were added as outlined below. In the [^{35}S]sulphate uptake experiments the medium also contained [^{3}H]PEG as a marker for extracellular tissue space and paracellular permeability. All media were prewarmed to 37° before being added to the tissue.

[^{35}S]*Sulphate uptake experiments.* Jejunal and colonic mucosal sheets were prepared according to Lauterbach [2]. Pieces of 0.2 cm^2 were mounted in flux chambers with 0.2 mL incubation medium on either side. The medium containing [^{35}S]sulphate and [^{3}H]PEG (0.3×10^6 cpm/mL each) was added alternately at the luminal side or blood side, while standard incubation medium was added to the non-radiolabelled side. Oxygen was supplied in a small bubbled stream at both sides, and incubation was performed at 37° for 20, 45 and 90 min.

Metabolism experiments. Mucosal sheets were prepared, mounted and incubated aerobically as described above. The medium containing [^{35}S]-sulphate (sp. act. 5×10^6 cpm/μmol = 5×10^6 cpm/mL) was added alternately to the luminal side or the blood side. Unlabelled 1-naphthol (50 nmol/mL) was either coadministered with [^{35}S]sulphate or added to the other side. In this way for each tissue four different experimental conditions were produced (compare Fig. 2).

Two series of experiments were carried out. In the main one, the tissue pool of inorganic sulphate was initially equilibrated with exogenous [^{35}S]-sulphate by preincubation for 30 min in the absence of 1-naphthol. The preincubation time was chosen as a result of the uptake experiments indicated above. The solutions on both sides were then withdrawn, and substituted with fresh [^{35}S]sulphate-containing medium at the preincubation side. The incubation was thereafter continued for a further 15 min in the presence of 1-naphthol on the appropriate side. The other series comprised experiments in which the preincubation step was omitted. Otherwise the procedure was as outlined above, except that the incubation time was prolonged to 45 min. The mean mucosal wet weights from the experiments were 12.34 mg for jejunal sheets (N = 49) and 5.34 mg for colonic sheets (N = 36).

Assay. In the [^{35}S]sulphate uptake experiments, the activities of [^{35}S]sulphate and [^{3}H]PEG in the luminal and blood side media and in the tissue at the end of incubation were determined by liquid scintillation counting (Betaszint BF5003 A, Berthold, Wildbad, Germany). The cellular concentration of [^{35}S]sulphate was calculated as described previously for other substrates [2], assuming a total extracellular space of 25% in jejunal sheets and 45% in colonic sheets.

In the metabolism experiments, the amounts of 1-naphthyl sulphate and of 1-naphthyl glucuronide in the samples were determined by HPLC and the specific activity of the sulphoconjugate was calculated following liquid scintillation counting. The specific activity of the [^{35}S]1-naphthyl sulphate was expressed as fractions (%) of the specific activity of the [^{35}S]-sodium sulphate administered which was taken as unity. The entire assay procedure can be summarized as follows: the mucosal sheets were homogenized, extracted overnight in 200 μL 50% methanol and centrifuged. The extracts were subjected to the same procedure as the samples of lumen and blood side media. To obtain the total activity of free [^{35}S]-sulphate and bound ^{35}S as a control, 50 μL aliquots of mucosal extracts and incubation solution were taken for scintillation counting. The overall concentration of 1-naphthyl sulphate (i.e. sum of labelled and unlabelled conjugate) and its specific radioactivity were determined.

Before assay by HPLC, the large excess of [^{35}S]-sulphate ions was removed by precipitation with barium chloride, to avoid contamination of the sulphoconjugate with inorganic [^{35}S]sulphate. To

achieve as complete a precipitation as possible, 100 μL of the supernatants were first enriched in sulphate ion by mixing with 25 μL 0.5 M unlabelled sodium sulphate solution. Twenty-five microlitres of 1 M barium chloride were then added, and the samples were centrifuged at 20,000 *g* for 1 hr at 4° (Sorvall RC-5B Refrigerated Superspeed Centrifuge, Du Pont Instruments, Wilmington, DE, U.S.A.) to obtain completely particle free supernatants. Remaining particles would otherwise rapidly cause obstruction in the HPLC system. Because of its unduly long retention time, the parent phenol was removed by extraction of 100 μL supernatant with 300 μL petroleum ether before the samples were injected on to the column.

Control experiments showed that: (1) barium chloride did not cause precipitation of 1-naphthyl sulphate. (2) The precipitation of [^{35}S]sulphate ion was complete in the above procedure (remaining activity 0.06%). (3) Petroleum ether extracted neither 1-naphthyl sulphate nor 1-naphthyl glucuronide. (4) 1-Naphthol was completely removed by the extraction. (5) There was a linear response between metabolite concentration and integrator signal (see below) within the actual range of the analysed samples.

The HPLC equipment consisted of the automatic injector WISP 712 (Millipore Waters, Milford, MA, U.S.A.) and the pump LKB 2150 (Pharmacia, Uppsala, Sweden). The chromatograms were monitored at 280 nm on an UV detector (Variable Wavelength Monitor, LKB 2151, Pharmacia). The chromatographic peaks corresponding to the 1-naphthyl metabolites were integrated and calculated by means of an integrator (SP 4100 Computing Integrator, Spectra Physics, San José, CA, U.S.A.). Analytical grade 1-naphthyl sulphate and 1-naphthyl glucuronide (Sigma, München, Germany) were used as standards. A C18 column (25 cm, μ Bondapak, 5 μm; Millipore Waters) equipped with a guard column (2 cm, LiChroprep RP-8, 25–40 μm; Merck) was used with a mobile phase of sodium phosphate buffer (pH 7.0, 50 mmol/L), acetonitrile and methanol (750:150:100, by vol.), and a flow rate of 1.2 mL/min. Under these conditions the retention times were 5.3, 9.7 and 49.5 min for 1-naphthyl glucuronide, 1-naphthyl sulphate and free 1-naphthol, respectively.

The eluate containing 1-naphthyl sulphate was collected in 1 min fractions (= 1.2 mL) for 4 min. These were mixed with a scintillation cocktail and counted. Sampling of the first fraction was begun 30 sec before the peak of 1-naphthyl sulphate began to appear. This and the second fraction contained most of the radioactivity. The last fraction was collected to ensure that all radioactivity had passed through.

Scintillation counting. Bray's scintillation cocktail [6] was used throughout. The degree of quenching was assessed by external standardization, and

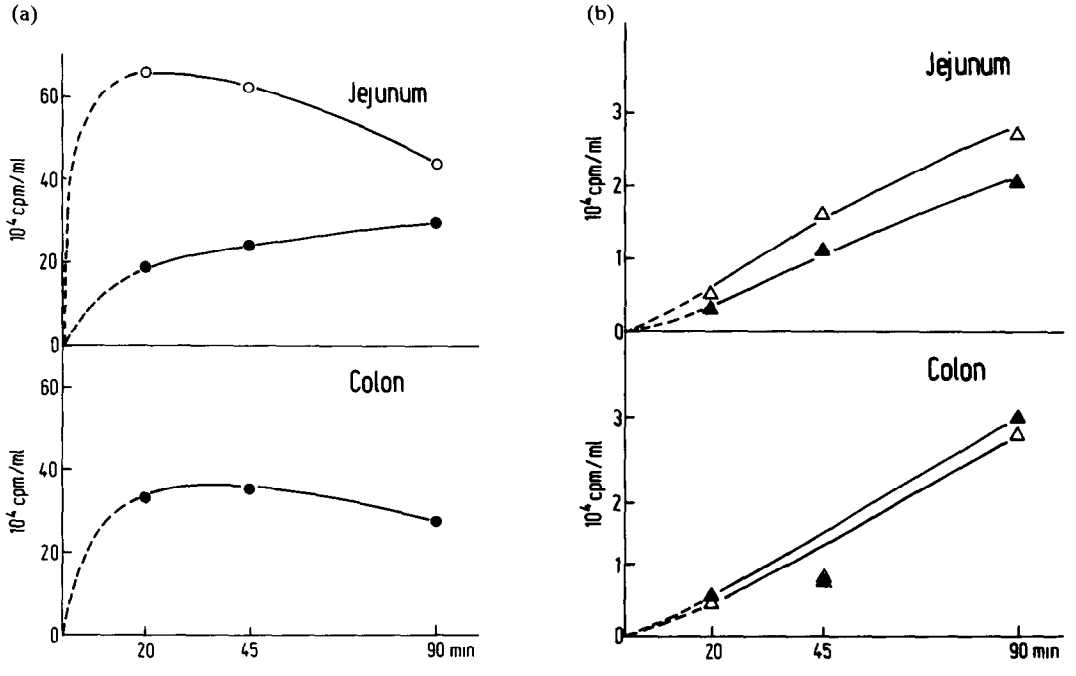

Fig. 1. Tissue labelling (a) and permeation (b) of [^{35}S]sulphate in the jejunum (upper parts) and colon (lower parts) as a function of time. Isolated mucosae of guinea pig intestine were incubated with 1 mM inactive sulphate ion on both sides. [^{35}S]Sulphate was administered unilaterally to the luminal side (open circles and triangles) and to the blood side (filled circles and triangles), respectively. [^{35}S]Sulphate dose: 0.3×10^6 cpm/mL. Ordinate: 10^4 cpm/mL intracellular space (a) or contralateral solution (b). After correction for extracellular space, there was no ^{35}S uptake left in the colon from the luminal side. Means of duplicates (range mean ± 5% for tissue labelling, mean ± 10% for permeation) are shown. Colon 45 and 90 min are single experiments.

Table 1. Distribution ratio of 1-naphthyl sulphate (1-NS) and 1-naphthyl glucuronide (1-NG): dependence on the experimental conditions

		1-NS	1-NG	1-NS	1-NG
		15 min, Lu:Bl		45 min, Lu:Bl	
JEJ	Adm. Lu	>167*	23.4 ± 3.2	44.4 ± 2.2	11.7 ± 1.8
		(4)	(4)	(8)	(8)
		15 min, Bl:Lu		45 min, Bl:Lu	
JEJ	Adm. Bl	>4.5† ± 0.7	7.5 ± 1.4	2.0 ± 0.4	6.9 ± 1.3
		(4)	(4)	(8)	(8)
COL	Adm. Lu	2.6 ± 0.3	16.1 ± 4.2	1.6 ± 0.3	17.4 ± 2.7
		(4)	(4)	(8)	(8)
COL	Adm. Bl	2.6 ± 0.4	24.7 ± 5.3	2.0 ± 0.2	21.9 ± 4.2
		(4)	(4)	(8)	(8)

Isolated mucosae of either guinea pig jejunum (JEJ) or colon (COL) were incubated for 15 or 45 min. 1-Naphthol (50 μM) was added either to the luminal (Adm. Lu) or blood side (Adm. Bl) and the appearance of both 1-NS and 1-NG on the luminal (Lu) and blood side (Bl) determined.

Distribution is expressed as the mean ratio ± SEM of 1-NS or 1-NG on the luminal and blood side (Lu:Bl; jejunum, luminal administration) or *vice versa* (Bl:Lu; all other conditions).

Number of experiments in parenthesis.

* In three out of four experiments the amounts on the blood side were below the detection level.

† In one experiment the amount on the luminal side was below the detection level.

corrections were made for variable quenching as well as for spillover of ^{14}C into the ^{3}H-channel and vice versa.

RESULTS

Labelling of ^{35}S-tissue pool

The time course of tissue labelling with radiolabelled sulphate and ^{35}S permeation of the mucosal sheet under the various conditions are shown in Fig. 1.

In the jejunum, higher values of cellular ^{35}S were obtained from the luminal side than from the blood side, and a maximum level was reached faster; probably within 20 min or less. When the radiolabelled sulphate was administered to the blood side, a maximum tissue level was still not attained after 90 min. Figure 1b shows that permeation of the mucosa was also faster when [^{35}S]sulphate was added at the luminal side.

In the colon the tissue took up [^{35}S]sulphate only from the blood side. The cellular concentration reached about 50% of the maximum value attained in the jejunum after 20 min with no further increase up to 90 min. In the luminal experiments, all radiolabelled sulphate in the tissue corresponded to the extracellular space measured simultaneously by PEG. The permeation of [^{35}S]sulphate ion through the tissue was, however, identical for the two routes of administration (Fig. 1b).

Jejunal metabolism following preincubation

The general picture of the metabolism of 1-naphthol (metabolism rate, glucuronide:sulphoconjugate formation, metabolite distribution to lumen and blood side, tissue accumulation) corresponded principally to that described previously [1]. Under the conditions used in this study (15 min incubation) the 1-naphthyl conjugates showed very markedly that they were preferentially transported to the side of 1-naphthol administration (Table 1). Even when 1-naphthol was incubated at the blood side the sulphoconjugate displayed a blood:lumen distribution ratio of at least 4:1, whereas incubation for 45 min in the previous [1] as well as present experiments gave mean ratios close to 1:1. Likewise 1-naphthyl glucuronide produced blood:lumen gradients consistently between 5:1 and 10:1 after the shorter, 15 min incubation when 1-naphthol was added to the blood side.

Utilization of the [^{35}S]sulphate for synthesis of the sulphoconjugate under the various conditions is shown in Fig. 2a. When 1-naphthol and [^{35}S]sulphate were co-administered at the luminal side, the sulphoconjugate showed about 70% of the activity of the precursor anion. The conjugate was found in a great excess at the side of precursor administration leaving a small, but likewise highly labelled (about 60%) amount in the tissue. The amounts on the blood side were in most cases too small to be determined (Table 1). When the radiolabel was offered at the blood side with 1-naphthol in the lumen, the specific activity of the main (luminal) fraction dropped drastically (to about 3%) as did the activity of the tissue fraction.

After co-administration of 1-naphthol and [^{35}S]-sulphate to the blood side, the specific activity was again high, but not quite as high as by luminal co-administration. The values were similar (in the range 50–60%) for all three sulphoconjugate fractions (lumen side, blood side, tissue). When 1-naphthol was maintained at the blood side, but the radiolabel was placed on the lumen side a complex picture was observed: the specific activity of the main fractions (i.e. tissue and blood side sulphoconjugate) dropped markedly, but less markedly than observed with

contraluminal [^{35}S]sulphate combined with luminal 1-naphthol. On the other hand the smaller, luminal fraction showed a dissimilar and higher specific activity than the two other fractions. (Compare with non-uniform labelling in the colonic experiments below.)

Colonic metabolism following preincubation

In accordance with previous findings [1] the metabolism and metabolite transport of 1-naphthol in the colon showed little dependence upon the side of drug administration. 1-Naphthyl glucuronide was distributed predominantly to the blood side (Table 1) as noted previously [1]. After a short incubation time (15 min) a preferential though less marked transport to this side was also noted for 1-naphthyl sulphate.

The incorporation of [^{35}S]sulphate may be summarized as follows (Fig. 2b): after co-administration of 1-naphthol and [^{35}S]sulphate to the lumen, a weakly labelled sulphoconjugate was obtained in all three fractions. At the same time the labelling was non-uniform: the lowest activity (less than 3%) was found in the main fraction, i.e. on the blood side, whereas the tissue fraction (9%) and the luminal fraction (10%) both displayed higher activities. However, adding the [^{35}S]sulphate to the blood side, but maintaining 1-naphthol in the lumen caused a high and similar degree of labelling in all three fractions (lumen 54%, blood 67%, tissue > 35%).

When 1-naphthol and [^{35}S]sulphate were co-administered to the blood side a specific activity of about 70% was obtained in all three fractions. If 1-naphthol and the [^{35}S]sulphate were applied at the blood side and lumen side, respectively, the specific activities of the various sulphoconjugate fractions dropped significantly, and were again non-uniform: the conjugate at the blood side had an activity as low as 3%, whereas significantly higher values were obtained in the luminal (about 20%) and tissue (about 25%) fractions.

Intestinal metabolism without preincubation

In the jejunum, incubation for 45 min showed the same general pattern concerning metabolism (sulphate:glucuronide ratio) and metabolite distribution (lumen:blood ratio) under the various experimental conditions as noted previously [1]. Compared to the values obtained after 15 min incubation, the conjugate distribution gradients tended to be less steep (Table 1). In effect, a more even distribution (close to 1:1) was observed for jejunal 1-naphthyl sulphate formed from the blood side, in contrast to the preferential transport to the blood side noted after 15 min incubation.

Table 2 summarizes the dependence of the specific activities of the luminal and blood side fractions of [^{35}S]1-naphthyl sulphate on the various experimental conditions. In agreement with the results of the preincubation experiments, co-administration of 1-naphthol and [^{35}S]sulphate caused the highest degree of labelling in the jejunum whether this took place at the luminal side or the blood side. Luminal 1-naphthol combined with contraluminal [^{35}S]sulphate lead to about a 90% loss in specific activity. The loss in activity was less pronounced (mean of about 50%) after contraluminal 1-naphthol and luminal [^{35}S]-sulphate.

In the colon, blood side [^{35}S]sulphate promoted the formation of highly radioactive 1-naphthyl sulphate irrespective of the side of administration of 1-naphthol. Under the two other conditions much more weakly labelled sulphoconjugate fractions were obtained.

Under all conditions involving incubation for 45 min the specific activities showed a considerable scattering. Non-uniform labelling within an experiment was not demonstrated with certainty. Both of these phenomena may have the same background as the less pronounced conjugate distribution gradients, as discussed below.

DISCUSSION

[^{35}S]Sulphate tissue labelling

The experiments undertaken in this study indicate two tissue pools of sulphate in the jejunum. They are accessible from the lumen side and blood side, respectively, and are further characterized by different capacities and above all by a dissimilar time course for [^{35}S]sulphate uptake. Furthermore, the jejunal experiments showed a more rapid transfer of [^{35}S]sulphate from lumen to blood than in the reverse direction, demonstrating that a net absorption of sulphate ion takes place in this part of the gastrointestinal tract, in accordance with jejunal studies in certain other species [7–9]. Most other studies have however been done in the ileum [10–15], which appears to represent the location of maximum absorption in various species [16]. The epithelial entry and exit mechanisms differ [17], involving sulphate/sodium-coupled influx at the brush border [11, 18–20] and sulphate/anion antiports at the basolateral membrane [19, 21, 22]. An additional sulphate/OH^- exchange mechanism in the brush border membrane was recently described in rabbit ileum [23] and pig jejunum [24].

The experiments in the colon gave clear evidence of one tissue pool only. This is accessible from the blood side and shows a time course for [^{35}S]sulphate uptake similar to the jejunal, "luminal" pool, but exhibits a different capacity. The identical curves for [^{35}S]sulphate permeation from lumen to blood and vice versa suggest that no net transport of sulphate ions takes place in the guinea pig colon.

The suggestion of compartmentation of the sulphate pool in intestinal tissue is interesting, and deserves a more thorough study. The purpose of the present experiments was, however, mainly practical, i.e. to establish those conditions under which the tissue pools were in equilibrium with exogenously added sulphate ion. All-in-all the ^{35}S-tissue concentration in the tissue was fairly constant between 30 and 45 min; therefore, a preincubation time with [^{35}S]sulphate of 30 min was chosen for the 15 min experiments with 1-naphthol.

Sulphoconjugation: 15 min versus 45 min incubation

In accordance with previous findings [1], in the jejunum *luminal* administration of 1-naphthol caused a rapid conjugation, whereas conjugation of

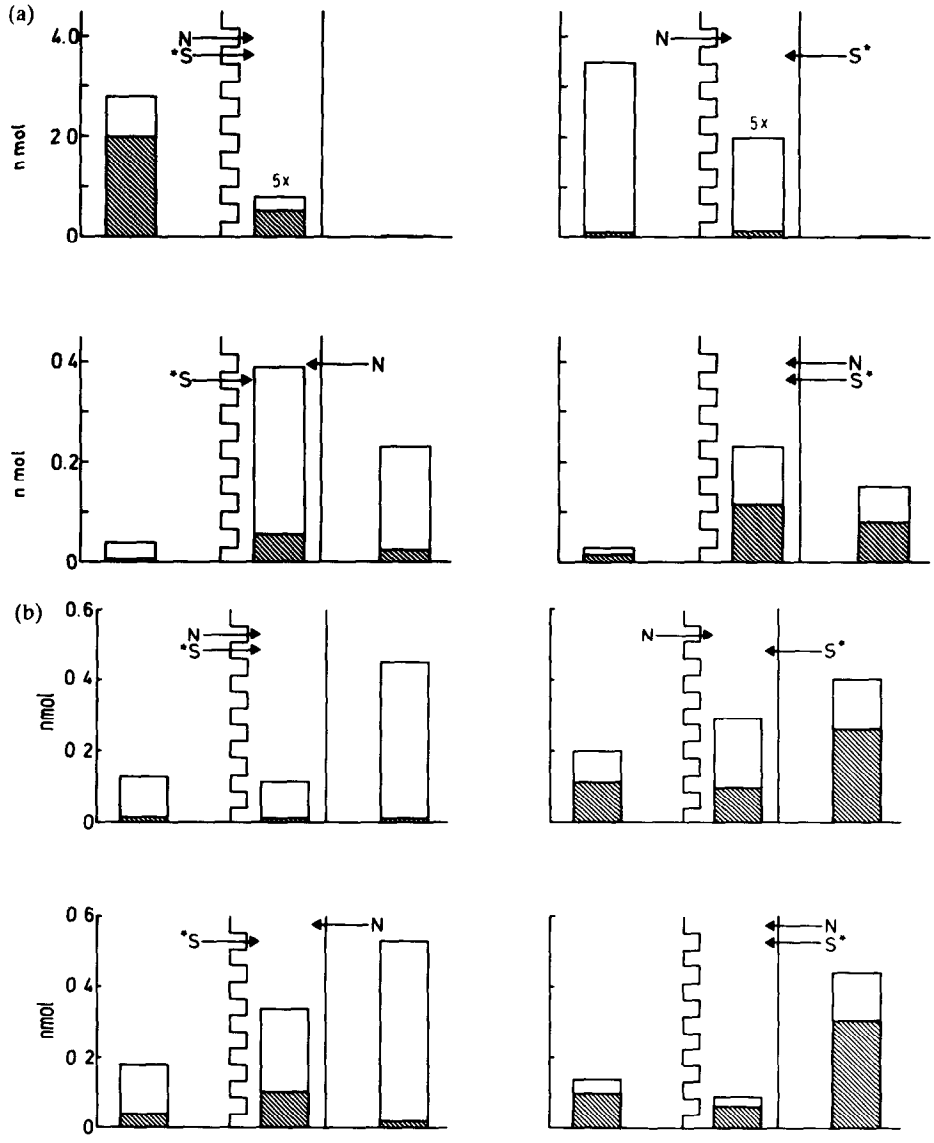

Fig. 2. Distribution pattern and ^{35}S activity of 1-naphthyl sulphate in the jejunum (a) and colon (b). Isolated mucosae of guinea pig intestine were incubated with 50 μM 1-naphthol (N) and [^{35}S]sulphate (S*) for 15 min following a 30 min preincubation with [^{35}S]sulphate. The heights of the columns show the amounts (nmoles) present at the luminal side (left), tissue (middle) and blood side (right) under the various administration conditions, which are indicated by arrows. The hatched part of the columns indicates the radioactivity of the sulphoconjugate; the ratio of hatched part to total column height (multiplied by 100) represents its specific activity in percentage of the specific activity of [^{35}S]sulphate administered. Note that in the jejunal experiments with luminal naphthol (a, upper part) the ordinate scale is reduced ten times and the tissue content magnified five times (with respect to that scale) because of the rapid metabolism and low tissue content under this condition. Each set of three columns represents one typical experiment. For the variability of the amount and distribution of 1-naphthyl sulphate compare left and right set (identical side of N administration) and Table 1. The relative specific activities varied by a factor of 1.1 or less between duplicates in fractions with values of 50% or higher and by factors of up to 4 in fractions with very low specific activities without any principal difference in the labelling pattern.

contraluminal 1-naphthol was low. With *luminal* administration, the metabolites were released predominantly to the lumen, while with *contraluminal* administration a less pronounced asymmetry was observed. In the colon the metabolism rate and pattern (glucuronide : sulphate ratio, data not shown here) and metabolite distribution (lumen : blood ratio; Table 1) seemed to be independent of the side of 1-naphthol administration.

Generally, the lumen : blood distribution gradients

Table 2. Dependence of the specific activity of [^{35}S]1-naphthyl sulphate (1-NS) on the side of administration of 1-naphthol (1-N) and [^{35}S]sulphate

1-N administration to		[^{35}S]sulphate administration to			
		Lumen		Blood	
		Specific activity of 1-NS (%)		Specific activity of 1-NS (%)	
		Lumen	Blood	Lumen	Blood
JEJ	Lumen	77.8 ± 12.0 (4)	62.4 ± 11.7 (3)	5.6 ± 2.0 (4)	6.7 ± 1.8 (4)
JEJ	Blood	34.2 ± 5.2 (4)	23.9 ± 5.1 (4)	52.1 ± 6.8 (4)	59.3 ± 11.0 (4)
COL	Lumen	8.8 ± 3.3 (4)	6.8 ± 2.6 (4)	58.2 ± 7.4 (3)	57.6 ± 9.9 (3)
COL	Blood	4.5 ± 0.4 (4)	3.2 ± 0.6 (4)	61.2 ± 5.6 (4)	83.6 ± 24.2 (4)

Isolated mucosae of guinea pig jejunum (JEJ) or colon (COL) were incubated for 45 min without preincubation. Initial concentration of 1-naphthol was 50 μM.

The activities of 1-NS released at the luminal or blood side are stated as percentage (mean ± SEM) of the specific activity of [^{35}S]sulphate in the incubation medium (5×10^6 cpm/μmol).

Number of experiments in parenthesis.

at 45 min incubation were less pronounced than at 15 min, as illustrated by the behaviour of jejunal sulphoconjugate formed from the blood side and colonic 1-naphthyl sulphate. Hence, it is obvious that the 45 min experiments tended to mask certain differences (concerning both the metabolite distribution and [^{35}S]sulphate incorporation), which are readily observed after 15 min. The discrepancies between the two experimental series may be caused partly by different design (preloading with [^{35}S]-sulphate or not), but have probably more to do with a short versus a long incubation time. With prolonged incubation the various fractions appear to mix, thereby causing a change in the distribution pattern of metabolites and radioactivity. One possible explanation is that mixing is occurring already during metabolism, i.e. that the precursors in spite of limited accessibility to some extent reach their contralateral compartment as time progresses. Another possibility is the diffusional mixing of the products of metabolism: a drug conjugate may thus (e.g. by the paracellular pathway) enter the luminal or blood side from the side into which it was secreted. In fact, recognition of these mixing phenomena lead to the procedure of preincubation with [^{35}S]sulphate followed by a short incubation with 1-naphthol.

The short incubation experiments thus may more truly reflect the inherent properties of the system under investigation. Therefore, discussion of the organization of the postulated "luminal compartments" and "systemic compartments" is based mainly on these experiments revealing outstanding differences in the labelling pattern under various conditions (compare Fig. 2). These differences were essentially confirmed by the results obtained in quadruplicate after 45 min incubation (Table 2). The above mentioned deviations from the 15 min results allow further conclusions on the properties of the compartment barriers.

Incorporation of [^{35}S]*sulphate in the jejunum*

With *luminal* administration of 1-naphthol co-administered [^{35}S]sulphate is incorporated into 1-naphthyl sulphate to a high degree, yielding specific activities of about 70%. Therefore, luminal sulphate represents the main exogenous source for sulphoconjugation of luminal 1-naphthol, as suggested by previous experiments [4]. This was confirmed by experiments where [^{35}S]sulphate was supplied from the blood side with 1-naphthol in the lumen: the very low specific activities obtained in this "reversed condition" demonstrate that the access of sulphate ions at the blood side to the metabolic "luminal compartment" is severely restricted.

When [^{35}S]sulphate was co-administered with 1-naphthol at the *blood side*, all 1-naphthyl sulphate fractions (lumen, blood side, tissue) again showed high though not quite as high specific activities (50–60% in both experimental series) as by luminal co-administration. As the fractions also were uniformly labelled, they evidently stem from one and the same metabolic source, in this case the "systemic compartment". This compartment depends to a high degree on exogenous sulphate supplied at the blood side. The experiments in the "reversed condition" (1-naphthol still on the blood side, but labelled sulphate in the lumen) indicated that in contrast to the "luminal compartment" contralateral sulphate ions are probably being utilized by the "systemic compartment" to a certain extent: the specific activity dropped markedly, but after a short incubation time was still about 10% in the two main fractions (i.e. blood side and tissue). Another significant feature was non-uniform labelling, since the luminal sulphoconjugate was more strongly labelled than the two other fractions. Possibly the luminal sulphoconjugate stems from a different metabolic source, i.e. from the "luminal compartment", which is slowly entered by 1-naphthol from the blood side. The still stronger, and dissimilar specific activities of luminal and blood side 1-naphthyl sulphate of 34% and 24%, respectively, after 45 min incubation support this notion. Likewise, the shift in the distribution ratio of 1-naphthyl sulphate in favour of the luminal side after prolonged incubation indicates migration of blood side 1-naphthol into the luminal compartment.

Strikingly, however, there is no clear indication so far that 1-naphthol, though a lipophilic compound, is able to enter the "systemic compartment" from the luminal side. Even after 1-naphthol incubation for 45 min in the lumen, only about 2% of the entire

1-naphthyl sulphate is found at the blood side though conjugate formed in the systemic compartment should have been released to the contraluminal side. Furthermore, even after 45 min both the luminal fraction and the minute amount of 1-naphthyl sulphate at the blood side have the same, very low specific activity of only 6%, when [^{35}S]sulphate is simultaneously added to the blood side.

Incorporation of [^{35}S]sulphate in the colon

Generally, [^{35}S]sulphate added to the blood side generates sulphoconjugates of high specific activities from 1-naphthol administered to either the luminal or blood side. The various fractions (lumen, blood side and tissue) show similar values indicating an origin essentially of the same compartment.

In contrast, luminal [^{35}S]sulphate is utilized only to a limited extent irrespective of the side of 1-naphthol administration. It is noteworthy, however, that the various fractions exhibit non-uniform labelling. For instance, 1-naphthyl sulphate transferred to the lumen is more strongly labelled than the conjugate on the blood side after luminal as well as after blood side administration of 1-naphthol. Hence, not all fractions can stem from the same compartment. Thus, the experiments provide evidence that the metabolism of 1-naphthol in the colon also takes place in two compartments.

Localization of the metabolic compartments

It might be suggested that the distinct differences in the handling of luminal and blood side 1-naphthol and [^{35}S]sulphate observed in the jejunum could simply be due to its crypt–villus organization and the ready access to the latter. In fact, very recent experiments [25] provided evidence that the histological correlate of the "luminal compartment" in the jejunum is made up by cells of the villus region, whereas the "systemic compartment" is represented by immature crypt cells. There are, however, a number of additional arguments that the compartmentation of intestinal drug metabolism cannot be explained simply by tissue geometry and different metabolic rates alone. First, 1-naphthol at the luminal side and [^{35}S]sulphate at the blood side do not combine. Neither a diffusional hindrance nor exhaustion of substrate can explain these phenomena. The extracellular space in the jejunal mucosa on the blood side is already equilibrated within 10 min [2]. Nevertheless, even prolongation of the incubation time to 45 min does not increase the specific activity of 1-naphthyl sulphate. Second, the existence of two separate compartments was demonstrated by independent means: unilateral preincubation with a hypertonic solution selectively damages either the luminal or systemic compartment. In the unaffected compartment neither a compensatory increase of e.g. glucuronidation of oestradiol nor a change in glucuronide distribution was observed [3].

The lack of villi in the colon might be regarded as a reasonable explanation for the inability to demonstrate a comparably pronounced compartmentation of phenol conjugation in this organ [1, 3, 26]. However, compartmentation of drug acetylation and acetylconjugate transport has been shown [3]. Moreover, for inorganic ion transport a differentiation has been demonstrated between colonic surface and crypts [27, 28] and even between individual membranes of surface and crypt cells [29, 30].

The dissimilar behaviour of the two compartments in both the jejunum and colon is therefore likely to reflect different properties of their cell populations with regard to sulphate supply on the one hand, and of drug uptake, metabolism rate and capacity, and metabolite transport, on the other.

Acknowledgements—The authors thank Mrs Erika Mannheim for her skilful technical assistance and Dr Keith Burdett, Manchester, for his linguistic help in preparing this manuscript. The financial support by the Minister für Wissenschaft und Forschung des Landes Nordrhein-Westfalen is thankfully acknowledged.

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