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A COMPARISON OF XENOBIOTIC METABOLISM IN CELLS ISOLATED FROM RAT LIVER AND SMALL INTESTINAL MUCOSA

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Abstract—The metabolism of several foreign compounds has been investigated in viable isolated liver and intestinal mucosal cells. Glucuronic acid and sulphate conjugation was observed in both cell preparations with respect to phenol. Conjugation of 1-naphthol was also observed in isolated intestinal cells. Acetanilide was the only metabolic product of aniline observed with intestinal cells. *N*-Acetylation was also found to be the major pathway of aniline metabolism in liver cells but *p*-hydroxylation followed by *O*-conjugation were also important reactions. Intestinal cells thus appear to be generally effective in conjugation reactions. However, in contrast to hepatocytes the intestinal cells were unable to form glycine conjugates from benzoic acid. The metabolism of 7-ethoxycoumarin and 7-hydroxycoumarin was investigated in isolated intestinal cells from control, 3-methylcholanthrene and phenobarbitone pretreated rats. Glucuronic acid and sulphate conjugation rates of 7-hydroxycoumarin appeared to be unaffected by these pretreatments whereas increases in 7-ethoxycoumarin *O*-deethylation were observed.

Knowledge of foreign compound metabolism in liver and extrahepatic tissues has derived largely from studies using tissue homogenates or microsomal preparations in which cofactors and substrates are generally added in excess to a non-physiological medium, and oxygen is made freely accessible. In the *in vivo* situation metabolism may be limited by membrane barriers to the free access of substrate to the metabolising enzymes, cofactor supply and competing reactions. The use of isolated cells may, therefore, be preferable to the use of tissue homogenates or fractions for many studies of foreign compound metabolism.

Isolated viable hepatocytes have been shown to be capable of carrying out a number of monooxygenase and conjugation reactions [1–7]. Although intestinal epithelial cells, prepared by a number of methods, have been used to study a variety of biochemical reactions [8–13], the biotransformation of foreign compounds by these cells has received only scant attention [14]. We have, therefore, chosen a number of model foreign compounds to compare major pathways of biotransformation in viable hepatocytes and intestinal epithelial cells. Benzoic acid was selected as substrate for the study of glycine conjugation (a mitochondrial reaction), phenol for sulphate (cytoplasm) and glucuronic acid formation (microsomal) and aniline for aromatic hydroxylation (microsomal) and conjugation (microsomal and cytoplasmic). The biotransformation of 7-ethoxy- or 7-hydroxy-coumarin was also studied in intestinal mucosal cells isolated from rats pretreated with phenobarbitone and 3-methylcholanthrene to examine whether typical inducers of drug metabolising enzymes in hepatocytes also induce in intestinal cells.

MATERIALS AND METHODS

Radiochemicals and chemicals

[U-^{14}C]Phenol (34 mCi/m-mole), [ring U-^{14}C]benzoic acid (36.3 mCi/m-mole), [U-^{14}C]aniline (60 mCi/m-mole) and 1-[1-^{14}C]naphthol (19.2 mCi/m-mole) were obtained from the Radiochemical Centre (Amersham, Bucks., England). The radiochemical purity of these compounds was checked by chromatography and found to be at least 98 per cent in all cases. All chemicals used in liquid scintillation counting were obtained from Packard Instruments Ltd. (Caversham, Bucks., England), except for *p*-bis-(*O*-methylstyril)-benzene (Bis-MSB) (Koch–Light Laboratories, Colnbrook, Bucks., England), 1:4-dioxan (Maybridge Chemicals, Tintagel, Cornwall, England) and toluene (Fisons, Loughborough, Leics., England).

Collagenase (type II), protease (type VII), hyaluronidase (type II), arylsulphatase (type III), bovine serum albumin (fraction V), saccharic acid 1:4 lactone, HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulphonic acid), 3-methylcholanthrene, *N*-acetyl-*p*-aminophenol, 1-naphthylsulphate and 1-naphthylglucuronide were purchased from Sigma Chemicals (Kingston-upon-Thames, Surrey, England). Sodium phenobarbitone was obtained from May and Baker (Dagenham, Essex, England) and ketodase (sulphatase-free, ox liver β-glucuronidase) was supplied by Warner and Chilcott (Eastleigh, Hants., England). Leibovitz L-15 medium with glutamine, tryptose phosphate broth and foetal calf serum were purchased from Gibco Biocult (Paisley, Renfrewshire, Scotland). 7-Hydroxycoumarin was

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supplied by Fluka A. G. (Buchs 59, Switzerland) and phenyl-β-D-glucuronide monohydrate was from Koch–Light Laboratories. 7-Ethoxycoumarin was synthesised according to the method of Ullrich and Weber [15] and benzoylglucuronide was isolated from the excretion products of a turkey dosed with benzoic acid using the method described by Baldwin *et al.* [16]. Other solvents, chemicals and authentic metabolites used in the metabolic studies were of Analar grade from British Drug Houses (Poole, Dorset, England).

Treatment of animals and isolation of hepatocytes and intestinal epithelial cells

Male Wistar albino rats bred in the University of Surrey Animal Unit were used (60–200 g).

For induction studies animals were pretreated by intraperitoneal injection, receiving either 0.5 ml corn oil (controls), or 3-methylcholanthrene in corn oil (25 mg/kg body wt) daily for four days; or 0.5 ml of 0.9% w/v sodium chloride solutions (controls); or phenobarbitone in sodium chloride solution (80 mg/kg body wt) daily for five days. The animals were allowed free access to food (No. 1 Laboratory Diet, Spillers Ltd., Croydon, Surrey, England) and water and they were killed by cervical dislocation 16–17 hr after the final injection.

Hepatocytes were prepared from rats (60–100 g) according to the method described by Fry *et al.* [3]. For metabolic studies the cells were suspended in L-15 Leibovitz medium [17] with glutamine, containing tryptose phosphate broth (10% v/v) and foetal calf serum (10% v/v). The cell density determined in an improved Neubauer Cell Chamber was $6–8 \times 10^6$ viable cells/ml and viability of the cells determined by trypan blue exclusion was in the order of 80–90 per cent.

Intestinal epithelial cells were isolated from the upper third of the small intestine. This section of the small intestine was excised from the animal and flushed with Kreb's calcium and magnesium-free phosphate buffer, pH 7.4 containing HEPES, 25 mM, glucose, 5 mM and bovine serum albumin 0.5% w/v (Kreb's CMF buffer). The intestine was everted, filled to slight distension with Kreb's CMF buffer and tied off at each end. The filled, everted lengths of intestine were incubated in Kreb's CMF buffer containing protease (10 units/ml) and EDTA (1 mM) in a 250 ml conical flask (one intestine/40 ml) for 30 min at 37° in a shaking water bath (50 cycles/min). The lengths of intestine were then removed to fresh Kreb's CMF buffer and shaken for a further 5 min at 37°. The combined cell suspensions were filtered through a layer of Bolting cloth (150 μm pore size, Henry Simon Ltd., Cheadle Heath, Stockport, England), and the filtrate centrifuged at approximately $200\,g_{av}$ for 1 min. The cell pellet was twice washed with ice-cold Kreb's CMF buffer, and resuspended in ice-cold Leibovitz L-15 medium (see above) to a concentration of approximately $7–9 \times 10^6$ cells/ml. Cells were counted in an improved Neubauer Counting Chamber (Gelman Hawksley, Lancing, Sussex, England) and cell viability as determined by trypan blue exclusion was in the order of 90–95 per cent.

Measurement of radioactivity

An L.K.B. "Wallac" 1210 scintillation counter was used. Counting efficiencies which were in the order of 70–90 per cent were determined from quench curves prepared using carbon tetrachloride as the quenching agent and n[1-^{14}C]hexadecane (The Radiochemical Centre) as the standard. Quench curves were prepared for each of the different scintillants and counting conditions used. Scintillant mixtures used included:

Dioxan-based scintillant—Naphthalene (60 g); 2,5-diphenyloxazole (PPO) (4 g); *p*-bis-2-(4-methyl-5-phenyl-oxazoyl)benzene (dimethyl POPOP) (0.2 g); methanol (100 ml); 1,2-ethanediol (40 ml) and 1:4 dioxan to give 1000 ml.

Toluene-based scintillant—PPO (5 g); BisMSB (0.25 g) and toluene (1000 ml).

Dioxan-based scintillant was used in the counting of fractions of extracts from cell incubations. Sections of silica gel thin-layer chromatograms were counted in toluene-based scintillants containing Cab-o-sil (5% w/v) while crystalline material from reverse isotope dilution analysis experiments was first dissolved in dimethylformamide (0.5 ml) and counted in dioxan-based scintillant. In all cases 10 ml of scintillant was used, and counting was carried out at 4° after storage at 4° in the dark to avoid possible errors due to chemiluminescence.

Autoradiograms of thin layer radiochromatograms were prepared using Kodak Blue Brand medical X-ray film. Autoradiography was for a period of 5–10 days and the resultant films were developed using Kodak Universal developer and fixed with "Kodafix" according to the recommendations of the manufacturers.

Metabolism studies using isolated cells

Metabolism of ^{14}C-compounds. Radiochemical compounds (5–10 μCi, 100 μM) were individually incubated with cell suspensions (5 ml) containing between 1×10^6 and 8×10^6 cells per ml at 37° in 50 ml conical flasks in a shaking water bath (50 cycles/min). Incubations were terminated by the addition of ice-cold acetone (1 ml). (Ice-cold acetone used at the concentration described caused an immediate uptake of trypan blue by all the cells, indicating severe cell damage.) The incubates were centrifuged to precipitate the denatured cellular materials and aliquots of the supernatant were taken for metabolite identification by thin layer chromatography, autoradiography and reverse isotope dilution analysis. The R_F values and methods of detection of ^{14}C-compounds used in this study and their metabolites are summarised in Table 1. Spray reagent (A) (see Table 1) was used to detect glucuronic acid conjugates while spray reagents (B) and (E) were used in the study of benzoic acid and aniline metabolism. Spray reagents (C) and (D) were used in the detection of phenol and its metabolites.

Quantitation of metabolite profiles was carried out by thin-layer chromatography of aliquots (20–40 μl) of the supernatant from the precipitated cells, followed by autoradiography. The areas on the chromatogram corresponding to the unchanged substrate and the authentic metabolites were located and compared with the corresponding spots on the autoradiogram. These areas were marked on the radiochromatogram, cut out or scraped off and placed into scintillation vials and counted as described. The radioactivity associated with each of these areas was expressed as a percentage of the total radioactivity recovered from the radiochromatogram.

Table 1. R_F values and colour reaction of ^{14}C-compounds (phenol, naphthol, benzoic acid and aniline) and their metabolites

Compounds	Solvent systems I	II	III	Colour reactions A	B	C	D	E
Phenol	0.96–1.00	0.98–1.00		—		yellow/red	blue	
Phenylglucuronide	0.44–0.49	0.46–0.51		blue		—	—	
Phenylsulphate	0.69–0.72	0.71–0.74		—		—*	—	
Quinolglucuronide	0.34–0.38			blue		yellow/red	blue	
1-Naphthol		0.93–0.98		—				
1-Naphtholglucuronide		0.57–0.62		blue				
1-Naphtholsulphate		0.71–0.77		—				
Benzoic Acid	0.90–0.96	0.70–0.75		—	—			—
Hippuric Acid	0.70–0.73	0.28–0.33		—	yellow			yellow
Benzoylglucuronide	0.35–0.42	0.46–0.52		blue	—			—
Aniline		0.91–1.00	0.74–0.86	—	—			N.D.
N-Acetyl-*p*-aminophenol		0.94–0.98	0.72–0.76	—	—			yellow
Acetanilide		0.94–0.98	0.52–0.61	—	—			—
N-Acetyl-*p*-aminophenylglucuronide		0.39–0.31		blue	—			yellow
n-Acetyl-*p*-aminophenylsulphate		0.62–0.66		—	—			yellow
p-Aminophenylsulphate		0.50–0.53	0 –0.1	—	yellow			yellow
p-Aminophenylglucuronide		0.12–0.15		blue	yellow			yellow
O-Aminophenylsulphate		0.71–0.73		—	yellow			yellow
O-Aminophenylglucuronide		0.40–0.42		blue	yellow			yellow

Solvent System: I butanol:acetic acid (glacial):water (4:1:1 v/v); II butanol:ethanol:acetic acid (glacial):water (3:1:0.1:1 v/v); III chloroform:methanol:acetic acid (glacial) (80:20:1) v/v).

A—Naphthoresorcinol in acetone (1% w/v) to which phosphoric acid (10% w/v) was added just before use (4:1 v/v) followed by heating of chromatogram to 140°.

B—*p*-Dimethylaminobenzaldehyde in EtOH (0.5% w/v) containing conc. HCl (1% v/v).

C—Freshly diazotized *p*-nitroaniline followed by 0.5 M KOH in EtOH.

D—Equal volume of freshly prepared potassium ferricyanide (1% w/v) and ferric chloride (2% w/v) followed by 2 M HCl.

E—0.5 M HCl followed by heating at 100° for 15 min, then spray with B.

— = No reaction.

N.D. = Not determined.

* = Yellow after a few minutes.

Identification of metabolites of [^{14}C]aniline was by t.l.c. and reverse isotope dilution analysis as described by Kao *et al.* [18]. Because of the volatile nature of phenol, thin layer chromatographic estimation as described above was not satisfactory. Therefore in the study of phenol metabolism by isolated cells, radioactivity associated with the areas on the chromatogram corresponding to the non-volatile conjugates was expressed as a ratio of sulphate to glucuronide. In addition aliquots of the cell supernatant were suitably diluted and extracted with ether. Ether extraction quantitatively removes unmetabolised phenol while little if any conjugate is extracted. The radioactivity in the ether and aqueous fractions was determined by liquid scintillation counting as described above. From the ratio of the non-volatile conjugates and the level of radioactivity associated with the ether and aqueous fractions, the relative proportions of phenol and phenol metabolites were determined.

Metabolism of 7-ethoxycoumarin and 7-hydroxycoumarin by isolated rat intestinal epithelial cells. Intestinal epithelial cell suspensions (5 ml) from control, phenobarbitone and 3-methylcholanthrene pretreated animals were incubated with 7-ethoxycoumarin (100 μM) in 25 ml conical flasks in a shaking water bath (50 cycles/min) at 37°. The reaction was terminated by the addition of ice-cold ether (7 ml) containing 1.5% v/v isoamyl alcohol. Under these conditions the highly fluorescent "unconjugated" 7-hydroxycoumarin was quantitatively extracted into the ether. The ether extracts (5 ml) were back extracted into 0.2 M glycine/sodium hydroxide buffer, pH 10.4, (3.5 ml). The fluorescence of the aqueous layer was determined at λ_{ex} 370 nm, λ_{fl} 450 nm using a Perkin–Elmer MPF-3 spectrofluorimeter and compared with appropriate standards prepared using 7-hydroxycoumarin.

Conjugate formation was determined by subsequent hydrolysis with β-glucuronidase and aryl sulphatase and followed by ether extraction and fluorimetric measurement of 7-hydroxycoumarin as described above. Due to limited cell yields from each animal the free 7-hydroxycoumarin and the glucuronide and sulphate conjugates were measured in a sequential manner in each sample. Although pure β-glucuronidase (ketodase) was available, the sulphatase preparation contained β-glucuronidase activities. Hence hydrolysis and estimation of glucuronides were carried out prior to determination of sulphate conjugates. For each enzymic hydrolysis the incubation medium was adjusted to pH 5.0 with 0.1 M acetate buffer. The samples were then incubated with ketodase (5000 Fishman units) or sulphatase (300 units) at 37° for 16–18 hr.

In some experiments the cell suspensions (5 ml) were incubated with 7-hydroxycoumarin (150 μM) in order to measure conjugation in the absence of mixed function oxidation. In these experiments unmetabolised 7-hydroxycoumarin was removed by ether extraction before the enzymic hydrolysis of conjugates and subsequent fluorimetric determination as described above.

RESULTS

Both intestinal and hepatocyte cell preparations metabolised phenol. In isolated hepatocytes, glucuronic acid and sulphate conjugates of phenol were the major metabolites; quinolglucuronide was also detected. A typical metabolite profile of phenol by isolated rat hepatocytes is illustrated in Fig. 1. Phenylglucuronide accounted for 75 per cent of phenol metabolites in isolated intestinal cells (see Table 2) and phenylsulphate for about 15 per cent. Traces of an unidentified metabolite (R_F 0.58 in solvent system I) were also detected by thin layer chromatography and autoradiography, in intestinal cell preparations, which were not detected in the hepatocyte incubations.

The glucuronide was also the major metabolite (~66%) of 1-naphthol in isolated intestinal cells with naphthol sulphate being the other major metabolite (results not shown).

The results of aniline metabolism summarised in Table 3 show that rat hepatocytes form similar metabolites to those produced *in vivo* [18]. Rat intestinal ephithelial cells also metabolised aniline, but to a much lesser degree. Reverse isotope dilution analysis demonstrated that the major metabolite of the intestinal cells was acetanilide; traces of *N*-acetyl-*p*-aminophenol were also detected.

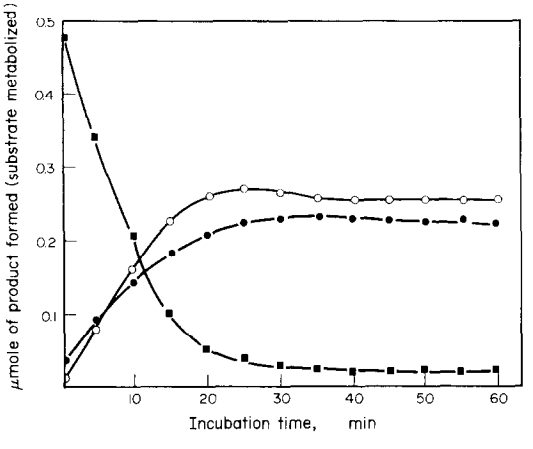

Fig. 1. Time course of the metabolism of [^{14}C]phenol in suspensions of rat hepatocytes. Viable hepatocytes (25 × 10^6 cells in 5 ml vol.) were incubated with [^{14}C]phenol (5 μCi, 100 μM) at 37° for periods up to 1 hr. At the times indicated aliquots of the cell supernatant were subjected to t.l.c., the chromatograms scraped and counted for radioactivity. ■: phenol; ●: sulphate; ○: glucuronide.

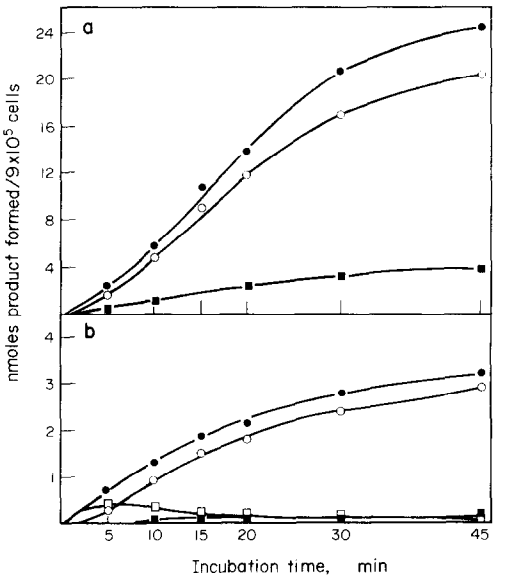

Fig. 2. The time course of metabolism of 7-hydroxycoumarin (a) and 7-ethoxycoumarin (b) in suspensions of small intestinal epithelial cells from control rats. Viable intestinal epithelial cells (approx 14 × 10^6 cells in 2 ml vol.) were incubated with (a) 7-hydroxycoumarin (100 μM) or (b) 7-ethoxy coumarin (100 μM) at 37° for periods up to 45 min. At the times indicated the suspensions were deconjugated, extracted with ether and the ether fraction extracted with glycine/NaOH buffer, pH 10.4. The various fractions obtained by this procedure were analysed fluorimetrically for 7-hydroxycoumarin. Cells were pooled from 4 animals. Results from (a) and (b) are paired, i.e. derived from the same pool of cells. Results represent the mean of two determinations. ●: total metabolites; ○: glucuronides; ■: sulphates; : free metabolites.

The metabolic fate of 7-hydroxycoumarin by isolated intestinal cells was similar to that seen with phenol or naphthol, the glucuronide being the major metabolite and the sulphate a more minor one (Fig. 2a). A lag phase of about 5 min was observed in these conjugation reactions which then continued linearly for about 20–30 min before plateauing. Using 7-ethoxycoumarin (100 μM) as substrate, this lag phase was also observed but only for the conjugation and not the oxidation reaction (Fig. 2b). The rate of glucuronidation was only 20 per cent of that observed when 7-hydroxycoumarin (100 μM) was used as substrate (Fig. 2a) but the sulphate/glucuronide ratio remained similar.

When 7-ethoxycoumarin (100 μM) was incubated

Table 2. Metabolism of [^{14}C]phenol in isolated rat intestinal mucosal cells

Incubation time (min)	Substrate/metabolite present: Phenol	Phenylglucuronide	Phenylsulphate
0	100.0	0.0	0.0
15	96.0	2.7	1.3
30	87.0	9.8	3.2
60	81.9	14.8	3.3

Incubation conditions are as outlined in Fig. 1. Results are expressed as per cent of total radioactivity recovered from the radiochromatogram. Cell concentration was 40 × 10^6 cells per 5 ml incubation volume.

Table 3. Metabolite profile of [^{14}C]aniline (100 μM) following incubation with isolated rat liver and intestinal cells for 1 hr

	Liver cells (%)*	Intestinal cells (%)
Aniline	0	92
N-Acetyl-*p*-aminophenol sulphate	20	nd.
N-Acetyl-*p*-aminophenyl glucuronide	14	nd.
p-Aminophenylsulphate	14	nd.
p-Aminophenylglucuronide	12	nd.
N-Acetyl-*p*-aminophenol	12	$\simeq$ 0.1
Acetanilide	13	8

*Results are expressed as % of radioactivity in the incubation medium corresponding to different metabolite. Incubation conditions are as outlined in Fig. 1, the cell concentrations being 35–40 $\times$ 10^6 cells per 5 ml incubation volume.

nd.—not detected.

with cells from the intestine of animals pretreated with phenobarbitone or 3-methylcholanthrene an increase in free and conjugated metabolites, as compared with untreated preparations, was observed and was more than 100 per cent (Fig. 3b). This increase in the rate of incubation was not observed when 7-hydroxycoumarin (100 μM) was incubated with cells from 3-methylcholanthrene-treated animals (Fig. 3a).

In the case of benzoic acid, essentially no metabolism (less than 1 per cent) by isolated intestinal cells was detected over an incubation period of 1 hr, whereas isolated hepatocytes showed an extensive metabolism of benzoic acid to give hippuric acid and benzoylglucuronide (Fig. 4).

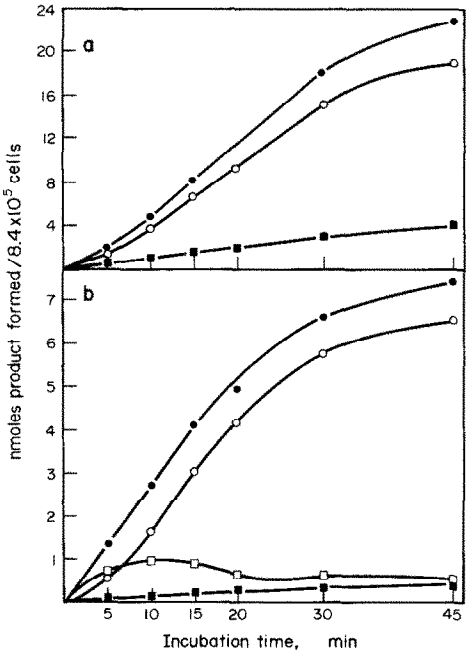

Fig. 3. Time course of the metabolism of 7-hydroxycoumarin (a) and 7-ethoxycoumarin (b) in suspensions of small intestinal epithelial cells from rats pretreated with 3-methylcholanthrene—all details as for Fig. 2. Animals received 3-methylcholanthrene (25 mg/kg in 0.5 ml corn oil) by intraperitoneal injection daily for 4 days and were killed 16 hr after the last injection.

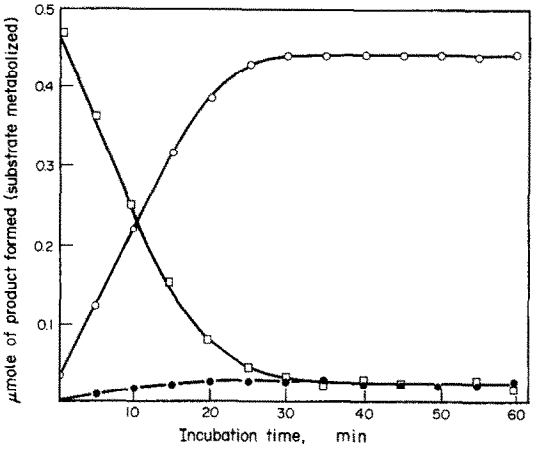

Fig. 4. Metabolism of [^{14}C]benzoic acid by isolated rat hepatocytes. Viable hepatocytes (30 $\times$ 10^6 cells per 5 ml volume) were incubated with [^{14}C]benzoic acid (5 μCi, 100 μM) at 37° for periods up to 1 hr. At the times indicated aliquots of the cell supernatant were subjected to t.l.c., the chromatograms scraped and counted for radioactivity. □: benzoic acid; ○: hippuric acid; ●: benzoylglucuronide.

DISCUSSION

Phenol was extensively conjugated with glucuronic acid and sulphate in both rat hepatocytes and intestinal wall cells. In the hepatocytes quinol was also formed whereas hydroxylation of phenol was not observed in the intestinal cell preparation.

The metabolism of 1-naphthol by isolated intestinal cells revealed a pattern of glucuronic acid and sulphate conjugation similar to that observed with phenol. No further hydroxylation products of 1-naphthol were detected. The isolated intestinal cell preparation tends to form proportionally more sulphate conjugates of phenols than is observed using intact gut preparations. The metabolism of phenol by the isolated perfused rat intestine has been reported by Powell *et al.* [19] to yield phenylglucuronide (95 per cent) and phenylsulphate (5 per cent) after 2 hr, while Bock and Winne [20] and Turner *et al.* [21] using intestinal loop preparations found negligible sulphate conjugation of 1-naphthol by the intestine. These differences may be related to the

fact that the conjugate assays were carried out at a much later time point using the intact intestinal preparations than in our studies involving isolated cells. Possibly depletion of PAPS or liberation of intestinal sulphatase with time could explain these discrepancies.

Only traces of free hydroxylated metabolites, glucuronide or sulphate conjugates of aniline were observed in isolated intestinal cells and this inability to detect the 4-hydroxylation of aniline in isolated intestinal cells confirms findings with microsomal preparations [22, 23]. However, the intestine showed a distinct capacity for acetylation of aniline to acetanilide. The acetylation of sulphanilamide by rat intestine [24] and the presence of a soluble isoniazid transacetylase in human intestinal mucosa [25] have been previously reported. Interestingly, the acetylation of aniline by isolated intestinal cells is seen in the presence of a considerable microsomal capacity to deacetylate acetanilide [23, 26]. This may be explained by the compartmentalisation of the deacetylase within the endoplasmic reticulum of the intact cells together with a low affinity of the enzyme for this substrate.

In contrast to the sulphate, glucuronic acid and acetyl conjugation activities, the ability of the intestinal mucosal cells to conjugate aryl acids with glycine appears to be very weak compared with the hepatocytes. This finding agrees with that of Strahl and Barr [27] who observed a very low rate of synthesis of hippurate by everted rat intestine. The rate of hydrolysis of added hippurate was insignificant, indicating that poor conjugating ability is the cause of the low hippurate levels. Irjala [28] reported that the duodenal glycine conjugation of benzoic acid is less than one seventh of that in liver.

7-Hydroxycoumarin conjugation with glucuronic acid and sulphate by the intestinal cells resembled that seen with phenol and naphthol. After 45 min incubation the glucuronide represented approximately 85 per cent of the total metabolites formed. This is in marked contrast to that found in hepatocytes [4] where almost 90 per cent of the product was 7-hydroxycoumarin sulphate. The rate of oxidation of 7-ethoxycoumarin was seen to be slower than the subsequent conjugation, indicating that *O*-dealkylation is the limiting step. However, unconjugated 7-hydroxycoumarin, the product of *O*-deethylation, was detected in the presence of its glucuronide and sulphate conjugates at all time points. Thus the monooxygenase and glucuronyltransferase (both of which are localized in the endoplasmic reticulum) do not appear to be tightly coupled.

The pattern of glucuronidation of 7-hydroxycoumarin suggests a lag phase in the initial 2–3 min of incubation. This lag was not seen during the *O*-deethylation of 7-ethoxycoumarin. This is unlikely to be due to inactivation of the conjugating system by the isolation procedure as conjugation proceeds readily at higher substrate concentration. A similar situation occurs in hepatocytes [5], which is thought to be due to the latency of glucuronyl transferase and its activation by its substrate. In contrast to the ring hydroxylation reactions investigated, in which the liver is much more active than the intestine, the estimated rate of *O*-deethylation of 7-ethoxycoumarin during the initial linear phase (9.6 nmoles/10^6 cells/hr) by intestinal cells is comparable to that for rat liver cell suspensions [3, 4]. The increase in response to the phenobarbitone and to 3-methylcholanthrene pretreatment is, however, much smaller (approx. 2-fold) than that observed for rat hepatocytes [4]. No induction of glucuronic acid or sulphate conjugation was detected.

It is apparent that the overall metabolic contribution of the gut will be most marked when the concentration of the drug in the lumen is low, as may occur following the ingestion of small amounts of environmental contaminants, or therapeutic agents which are slowly released to the intestinal wall, particularly if they already contain a group which is suitable for conjugation. Furthermore, it is apparent that the major route of substrate conjugation taken by both hepatocytes and intestinal mucosa cells is markedly dependent on the substrate under study and thus studies using a large range of model substrates are essential for accurate prediction of the true conjugative ability of these cells.

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