Cutaneous metabolism – an historical perspective

Simon Wilkinson and Faith Williams
Medical Toxicology Centre
Newcastle University, UK

Health Protection Agency

Skin Metabolism Meeting CR Europe
20th May 2011

Newcastle University

Overview – an historical "snapshot"

- Importance
- Approaches used
- Expression, activity, functional consequences for Phase I enzymes
  - Localisation
- Phase II enzymes
  - As above
- The future

Importance of cutaneous metabolism

- Influence on toxicity
  - Activation of chemical agents to toxic metabolites
  - Skin sensitisation – metabolic involvement
  - Detoxification through the dermal route
  - Enhancement of absorption by ester hydrolysis e.g. Fluazifop butyl

Importance of cutaneous metabolism

- Influence on drug delivery
  - Ester (and other) pro drugs
  - Cutaneous side effects
  - Drug-drug interactions
- Importance for skin physiology
  - Desquamation
  - Other endogenous substrates

Approaches used

- Gene expression (mRNA)
- Protein expression (immunological)
- Enzyme activity in homogenates and subcellular fractions using classical probe substrates
- Functional studies – consequences (products) of metabolic activity
  - E.g. Detection of epoxides
- Localization – immunological, ISH, activities
- Whole skin, homogenates, isolated cells

Specific activities of drug-metabolizing enzymes in different cell fractions from control and β-NF-treated mouse skin

Cells were isolated by a 60-min incubation with trypsin (0.1% in HPBS) and separated on a discontinuous metrizamide gradient (0, 10 and 20% in HPBS). Activity in the enzymes was measured as described under "Materials and Methods." β-NF animals were pretreated by topical administration of β-NF (1.25 mg/mouse in 100 μl of acetone 16 to 18 hr before the animals were killed).

Cell Preparation	7-Ethoxycoumarin O-Deethylase		Benzo(a)pyrene Hydroxylase		UDP-glucuronosyl transferase (4-MU)		GSH-S-Transferase (CDNB)	
	Control	β-NF	Control	β-NF	Control	β-NF	Control	β-NF
	pmol/min/mg DNA		*pmol/min/mg DNA*		*nmol/min/mg DNA*		*nmol/min/mg DNA*	
Original	228 ± 34[a]	4,286 ± 880[b]	118 ± 61	1,327 ± 737[b]	5.2 ± 0.9	15.1 ± 1.0[b]	316 ± 40	482 ± 106[b]
Sebaceous	1,051 ± 221	10,219 ± 4,499[b]	2,377 ± 1,164	5,157 ± 2,777[b]	39.3 ± 13.8	54.4 ± 27.5	1,399 ± 802	1,131 ± 517
Basal	175 ± 24	2,759 ± 522[b]	735 ± 360	3,011 ± 1,789[b]	13.7 ± 0.8	27.2 ± 14.7	399 ± 190	862 ± 350[b]

[a] Each value represents the mean ± S.D. ($N = 3$).
[b] $P < .05$ *vs.* control.

Coombes et al(1983) J Pharmacol Exp Thera, 225: 770-776

Aldrin epoxidation by rat skin during percutaneous absorption *in vivo*

M. J. GRAHAM, FAITH M. WILLIAMS & M. D. RAWLINS
The Wolfson Unit of Clinical Pharmacology, University of Newcastle upon Tyne, Newcastle upon Tyne

In vitro studies have shown that rat skin is able to metabolise aldrin to dieldrin (Rawlins *et al.*, 1983). The present investigation was designed to study whether this occurs *in vivo* following topical application.

Animals received aldrin (10 mg kg^{-1}) either applied topically to 6 cm^2 of dorsal skin (in dimethyl sulphoxide) or intraperitoneally (in ethanolic saline). Blood samples were withdrawn from the tail vein at intervals and aldrin and dieldrin concentrations measured by gas-liquid chromatography with electron-capture detection (Williams *et al.*, 1982).

After i.p. administration of aldrin, peak whole blood concentrations of aldrin (890.0 ± 92.0 ng ml^{-1}, mean ± s.e. mean) were achieved at 10 min. Peak dieldrin concentrations (207.0 ± 18.0 ng ml^{-1}) were observed after 2.5 h, and decreased to 148.0 ± 9.0 ng ml^{-1} at 7 h. After topical administration, peak aldrin concentrations (44.1 ± 11.6 ng ml^{-1}) were observed after 1 h. Dieldrin concentrations rose gradually to 48.0 ± 15.0 ng ml^{-1} at 7 h.

In a further study, groups of female rats ($n = 4$) were killed at 1, 2, 3, 5 and 7 h following topical applications to 6 cm^2 of dorsal skin or intraperitoneal injection of aldrin (10 mg kg^{-1}). Aldrin and dieldrin concentrations were measured in skin at the site of application and at a remote site (ventral skin). After i.p. administration the concentrations of aldrin and dieldrin were similar in dorsal and ventral skin at all times. After topical application, dieldrin concentrations were higher in dorsal skin than in ventral skin at all times except at 1 h. The concentrations at 7 h are shown in Table 1.

These studies indicate that aldrin undergoes metabolism to dieldrin in rat skin during percutaneous absorption.

M. J. Graham is an MRC student.

Table 1 Concentrations of aldrin and dieldrin

$n = 4$	*i.p.*		*Topical*	
	Dorsal skin	*Ventral skin*	*Dorsal skin*	*Ventral skin*
Aldrin (nmol g^{-1})	1.78 ± 0.27	2.03 ± 0.47	511.3 ± 53.7	2.25 ± 1.42
Dieldrin (nmol g^{-1})	4.38 ± 0.34	3.9 ± 0.71	7.56 ± 2.0	2.44 ± 1.12

Rawlins, M. D. *et al.* (1983). *Br. J. Pharmac.*, **80**, 712P.

Williams, F. M. *et al.* (1982). *Biochem. Pharmac.*, **31**, 3701–3703.

Table 1. Mouse skin and liver microsomal mixed-function oxidase activities.

Substrate	Specific activity (pmol/mg per min)		Ratio of skin/liver × 100
	Liver	Skin	
Benzo[α]pyrene	209 ± 28	8·2 ± 0·5	3·9
Diphenyloxazole †	279 ± 6·0	5·7 ± 0·6	2·0
Ethoxyresorufin	267 ± 13	40 ± 3·6	15
Aldrin	402 ± 33	4·0 ± 0·4	1·0
Coumarin	27·3 ± 3·0	0·13 ± 0·03	0·5
Methoxycoumarin	479 ± 50	3·9 ± 0·2	0·8
Ethoxycoumarin	932 ± 88	7·2 ± 0·7	0·8
Propoxycoumarin	628 ± 82	3·4 ± 0·4	0·5
Butoxycoumarin	267 ± 32	2·5 ± 0·3	0·9
Cytochrome P-450 ‡	960 ± 20	n.d.	—

Values are the means ± S.E.M. of four separate experiments. Each experiment utilized microsomes pooled from 6–10 animals. Measurements of enzyme activity were performed as described in *Materials and methods*.

n.d., not detected

†Fluorescence units/mg per min.

‡pmol/mg.

Rettie AE, Williams FM, Rawlins MD (1986)
Xenobiotica, 16: 205-211

Table 2. Mouse skin co-factor requirements for mixedfunction oxidase activity.

	Enzyme activities	
	Aldrin epoxidase	Ethoxyresorufin O-dealkylase
Complete system	100	100
Complete system less NADH	81 ± 2	100 ± 1
Complete system less NADH and NADPH	4 ± 1	0
Complete system plus CO (3 : 1;air : CO)	14 ± 1	12 ± 1
Complete system plus N_2	11 ± 4	4 ± 2
Complete system plus DMSO (4%)	90 ± 5	101 ± 4
Complete system plus metyrapone (1 mM)	56 ± 4	40 ± 6
Complete system plus α-naphthoflavone (0·5 mM)	94 ± 3	0

Assays were carried out as described in *Materials and methods* and values are the means ± S.E.M. of three separate experiments. Each experiment utilized microsomes pooled from 6–10 animals. Enzyme activities are expressed as a percentage of activity in the complete incubation system which contained 0·8 mM NADPH and NADH and up to 1 mg/ml microsomal protein in 0·1 mM KCl–phosphate buffer, pH 7·5.

Rettie AE, Williams FM, Rawlins MD (1986)
Xenobiotica, 16: 205-211

Aldrin

- Aldrin was metabolised to dieldrin in viable rat skin preparations, but the parent compound and metabolite remained in the skin
- Aldrin was absorbed in receptor fluid with ethanol:water receptor fluid, but viability was not maintained.
  - McPherson et al (1991) Arch Toxicol 65: 599-604

Cytochromes P450

- Cytochromes P450
  - mRNA: 1A1, 1B1, 2B6, 2E1, 3A4, 3A5
  - Protein: as above in humans, 1A1/2 in mice, 1A1, 1B1, 2E1, 3A1 in SD rats. 3A "constitutive" in HEKs, aromatase and 3A in ex vivo human skin/sebaceous glands
  - AHH, EROD, PROD activities detected
  - 1A1 in epidermis, others localised to basal layer, sebaceous glands, hair follicle cells

CYP activity in vivo

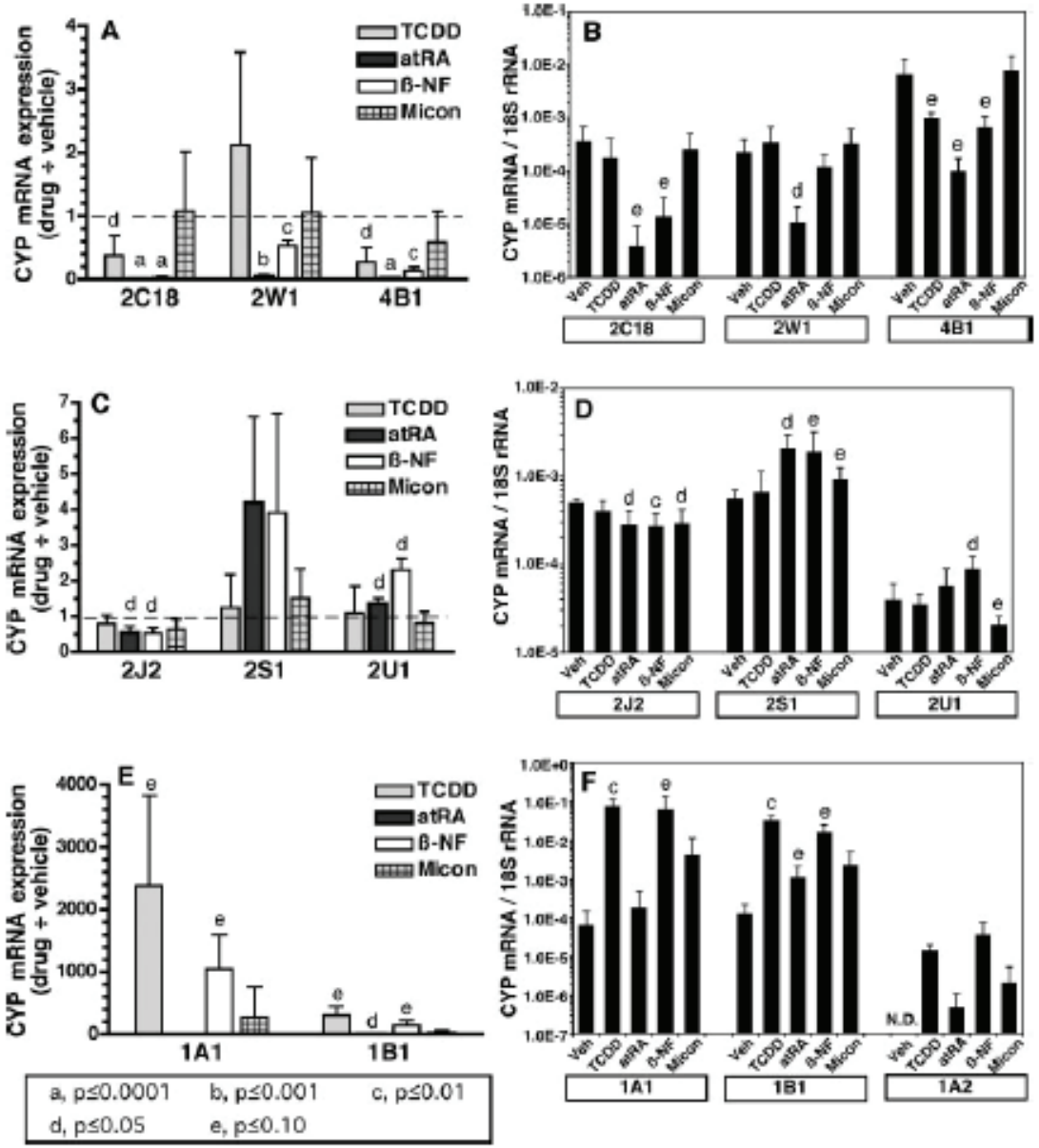
- Human skin biopsies (healthy volunteers and psoriasis patients) Smith et al. 2003
  - 1B1, 1A1, 2S1 consistently expressed
  - 2E1 in some individuals, higher in lesional skin (as was 2S1)
  - 2S1 highly induced with coal tar treatment
- Yengi et al 2003
  - Main isoforms expressed were 1B1, 2B6, 2D6, 3A4 (2C18, 2C19, 3A5)

CYP2 Expression

Table 2
List of human *CYP2* gene subfamilies—summary of those containing functional member loci and evidence for cutaneous versus epidermal expression

CYP2 gene subfamilies	Functional loci	Cutaneous expression	Epidermal expression	Member loci expressed
CYP2A	+	+	–	*CYP2A6, CYP2A7*
CYP2B	+	+	+	*CYP2B6*
CYP2C	+	+	+	*CYP2C9, 2C18, 2C19*
CYP2D	+	+	–	*CYP2D6*
CYP2E	+	+	+	*CYP2E1*
CYP2F	+	–	–	
CYP2G	–			
CYP2J	+	+	+	*CYP2J2*
CYP2R	+	+	+	*CYP2R1*
CYP2S	+	+	+	*CYP2S1*
CYP2T	–			
CYP2U	+	+	+	*CYP2U1*
CYP2W	+	+	+	*CYP2W1*

Du et al. (2004) Toxicol Appl Pharmacol 195: 278-287



Response to retinoic acid or AhR ligands is modulated by differentiation status – A and B are genes upregulated by differentiation specific factors, C-F are weakly regulated or down regulated by differentiation specific factors.

Du et al., 2006 J Pharmacol Exp Thera 319: 1162-1171

Esterases

- Histochemical and functional detection of esterase activity in numerous skin species.
- Easily released during homogensation
- Cytosolic generally higher than microsomal
- detected in all layers of skin (but less in dermis)
- Importance during percutaneous absorption identified e.g. Fluazifop butyl

Carbaryl

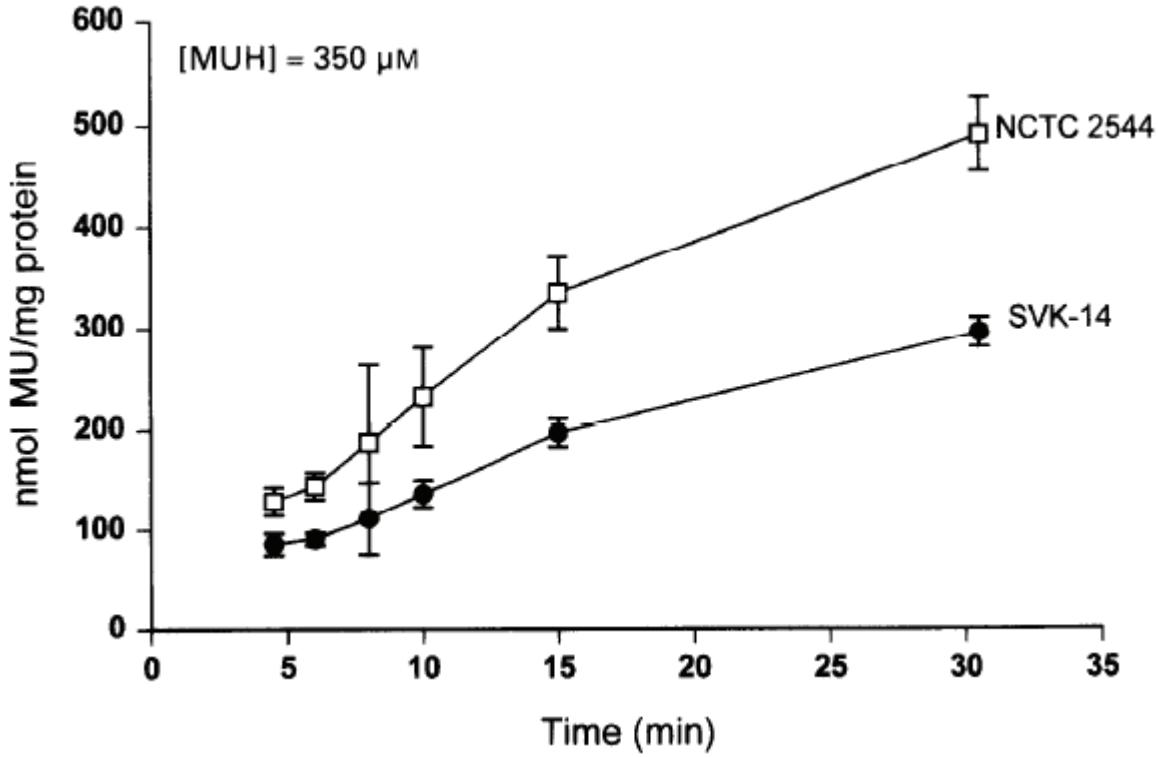
- In rat liver, hydrolysis, ring hydroxylation and conjugation detected
- In rat skin (post mitochondrial fraction), only hydrolysis and conjugation detected.
- No metabolism detected during percutaneous absorption
  - McPherson et al (1991) Arch Toxicol 65: 594-598

Hydrolytic activity in rat skin cf lung and liver microsomes/cytosol

Vmax	Liver	Lung	Skin
Fluazifop Butyl	m 6.2	m 0.4	m 0.02
(μmol/min/g)	c 6.8	c 1.5	c 0.4
Carbaryl	m 2.1	m 1.6	m 0.2
(nmol/min/g)	c 6.7	c 1.4	c 0.5
Paraoxon	m 330	m 2.0	m nd
(nmol/min/g)	c nd	c nd	c nd

Phenylacetate activity detected in all tissues
Hydrolysis of FB was inhibited by paraoxon and bisnitrophenol phosphate
McCracken et al (1993) Biochem Pharmacol 45: 31-36

4-MUH hydrolysis in cell lines



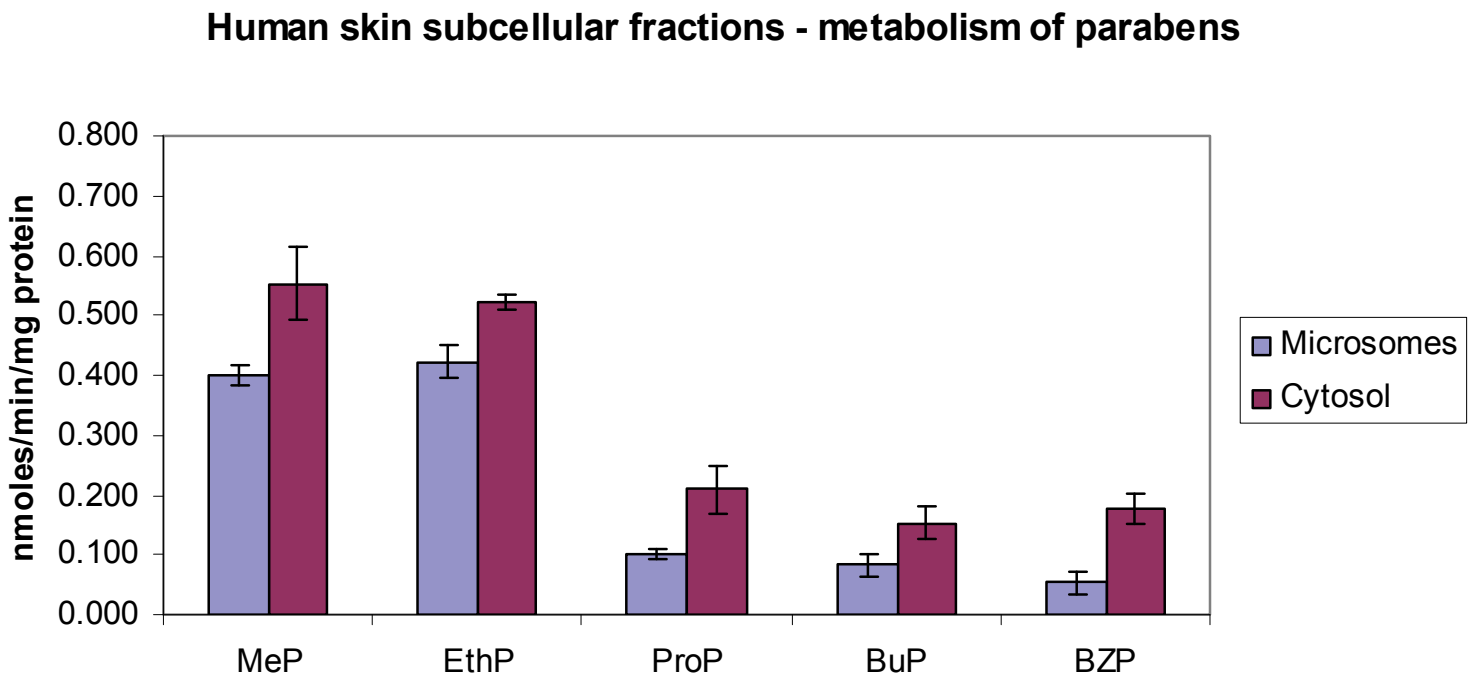
Barker and Clothier (1997) Toxicol in Vitro 11:637-640

Metabolism of parabens

- Two main cutaneous esterase activities identified by Lobemeier et al (1996)
  - Prominent activity preferred methyl paraben, activity decreased with chain length
  - Second activity in subcutaneous fat
  - Activity in HaCat extracts preferred butyl paraben, activity decreased with decreasing chain length

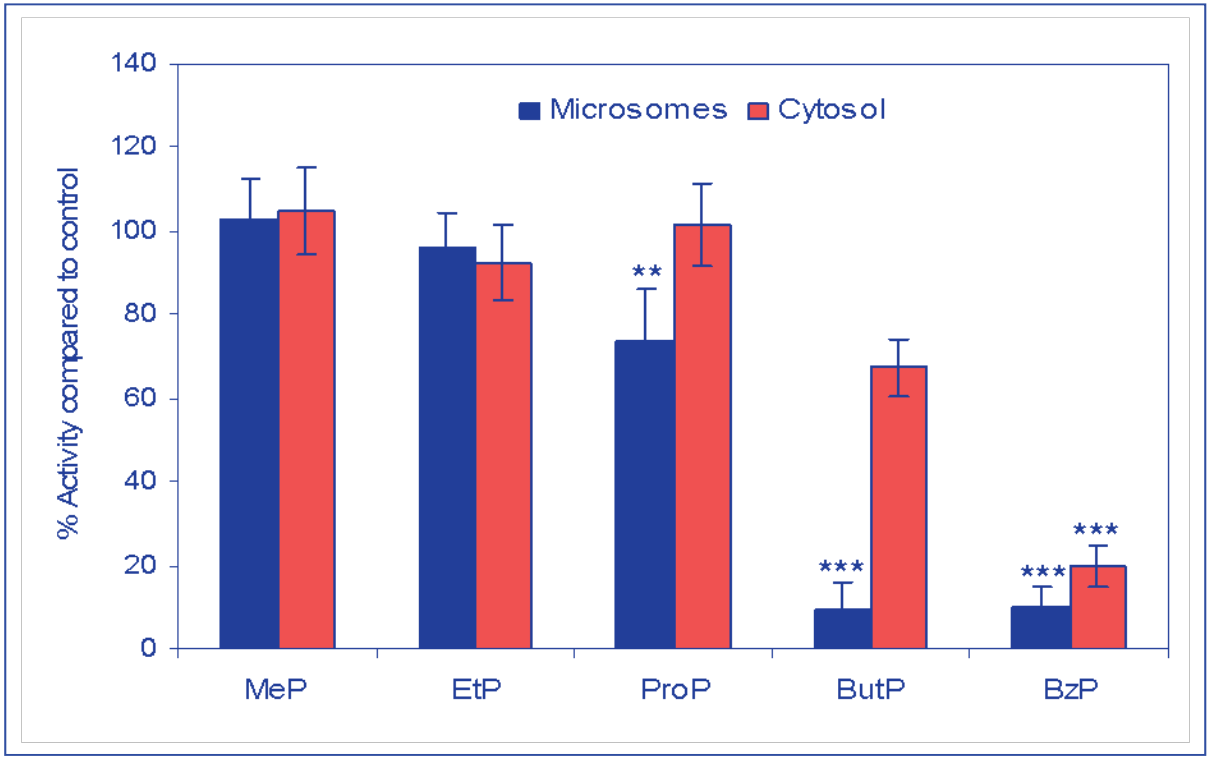
Lobemeier et al. 1996 Biol Chem 377:647-651

Esterase expression and activity



Jewell et al 2007 Toxicol Appl Pharmacol 225, 1-22

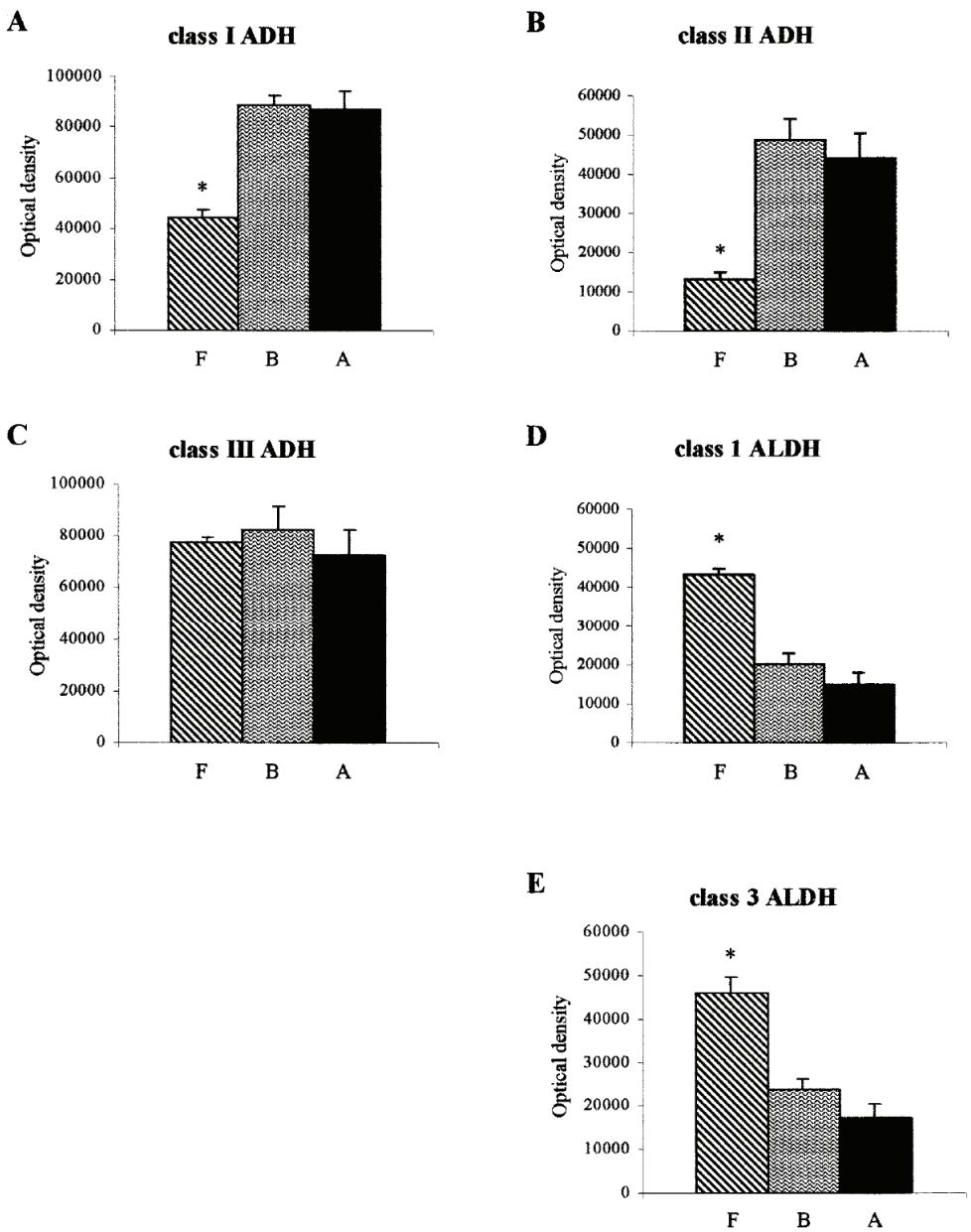
Inhibition of paraben hydrolysis by loperamide (hCE2 inhibitor)



Jewell et al 2007 Toxicol Appl Pharmacol 225, 1-22

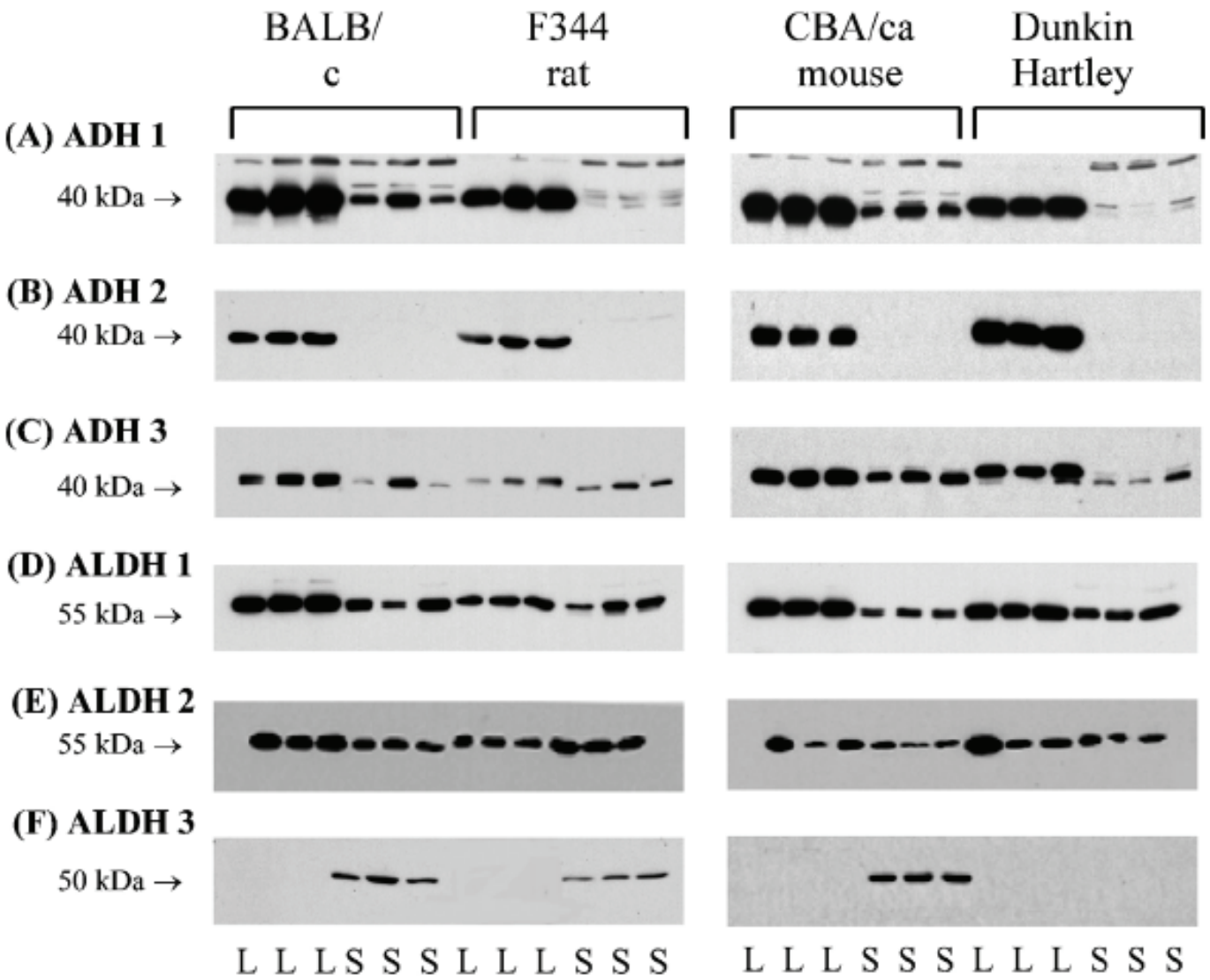
Alcohol and Aldehyde Dehydrogenase

- Catalytic activities (alcohol to carboxylic acid) reported in intact skin and subcellular fractions
- Protein expression (ADH1, 2 and 3; ALDH 1 and 3) detected in skin by Western blotting
- Histochemical localisation to epidermis and appendages. Little ADH2 detected.



Semiquantitative densitometric analysis of immunoreactive bands resulting from Western blot analysis of human skin cytosol with class specific ADH and ALDH antisera.

Cheung et al. (1999) Biochemical and Biophysical Research Communications **261, 100–107**



Cheung et al. (2003) Toxicology 184: 97-112

Kinetic constants for ethanol oxidation catalysed by ADH in skin and liver cytosol

	V_{max}		K_m		V_{max}/K_m	
	Liver	Skin	Liver	Skin	Liver	Skin
Human (B)	–	0.3±0.1	–	0.4±0.1	–	0.8
Human (A)	–	0.4±0.2	–	0.4±0.2	–	1.2
Mouse (M)	16.7±4.6	1.1±0.2	1.5±0.1	9.2±7.7	11.1	0.1
Mouse (F)	10.5±4.8	1.2±0.4	1.5±0.7	1.0±0.7	7.3	1.2
Guinea-pig (M)	6.0±2.4	0.6±0.8	1.1±0.4	0.2±0.2	5.5	3.1

V_{max} values are expressed as nmoles NADH/mg protein/min and K_m in mM. Kinetic constants were calculated with the ENZPACK program (Biosoft, Cambridge, UK) using the Direct Linear plot method. Results are mean ±S.D. for female human breast skin (B, $n = 7$), female human abdomen skin (A, $n = 5$), male and female BALB/c mice (M and F, $n = 3$) and male guinea-pig ($n = 3$).

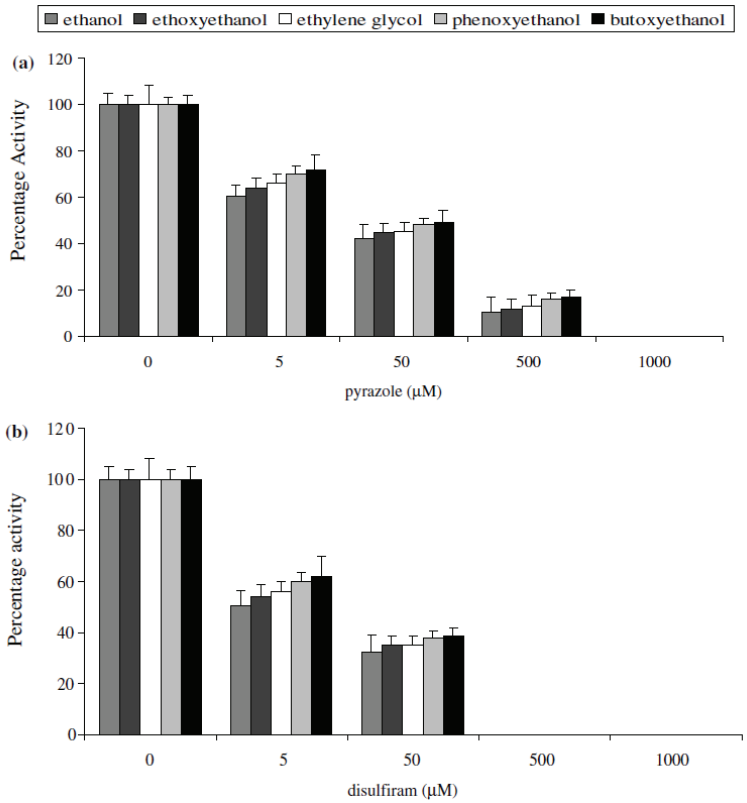
Cheung et al. (2003) Toxicology 184: 97-112

Table 1 Kinetic parameters of alcohol and glycol ether metabolism by rat liver and skin cytosolic alcohol and aldehyde dehydrogenases. K_m (mM) and V_{max} (nmol NADH formed/min/mg protein) represent means ±SEM for five animals

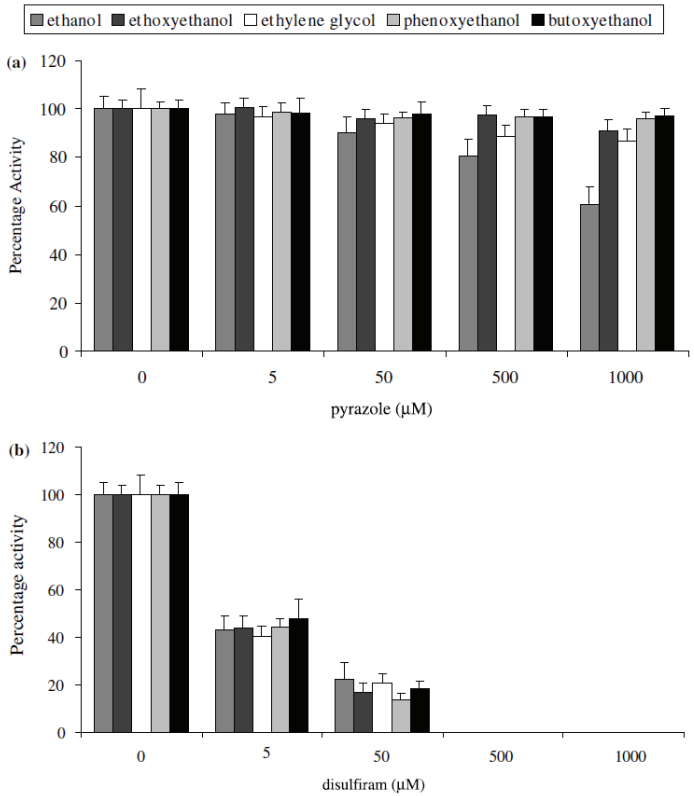
Substrate	Liver cytosol		Whole skin cytosol		Dermatomed skin cytosol	
	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
Ethanol	0.39±0.02	40.53±2.02	0.86±0.06	2.06±0.10	0.86±0.04	3.90±0.19
2-Ethoxyethanol	0.51±0.03	17.46±0.87	0.77±0.05	2.69±0.13	0.76±0.04	5.33±0.26
Ethylene glycol	0.67±0.03	11.73±0.58	0.72±0.04	6.28±0.31	0.72±0.05	11.95±0.59
2-Phenoxyethanol	0.92±0.06	11.42±0.57	0.70±0.04	12.91±0.64	0.69±0.06	24.52±1.22
2-Butoxyethanol	1.49±0.27	3.27±1.29	0.56±0.03	15.54±0.77	0.55±0.28	29.51±1.47

Lockley et al. Arch Toxicol (2005) 79: 160–168

RAT LIVER cytosol



RAT SKIN cytosol



Lockley et al. Arch Toxicol (2005) 79: 160–168

Other oxidoreductases

- Flavin-containing monooxygenases
  - Very few reports in skin
- NAD(P)H Quinone oxidoreductases
  - Detected in rodent epidermal cytosol at higher levels than in liver
  - Inducible by substrates and 3-MC
  - Easily detectable and inducible in keratinocytes in culture

Phase 2 Enzymes

- Glutathione transferases (mainly pi isoform)
- Sulphotransferases (isoforms?)
- Glucuronyl transferases – range of substrates reportedly conjugated
- N-acetyl transferases (NAT-1)
  - Rapid N-acetylation of aromatic amines

Table I. GST Activity in Rodent and Human Skin Cytosol[a]

Substrate	Rat	Mouse	Human
1-Choro 2,4-dinitro-benzene[b]	59.50 ± 3.91	54.07 ± 4.52	25.82 ± 1.95
Leukotriene A_4[c]	27.33 ± 2.80	12.32 ± 1.13	5.90 ± 0.42
Ethacrynic acid[b]	3.05 ± 0.22	15.50 ± 1.50	5.02 ± 0.41
Benzo(a)pyrene 4,5-oxide[a]	0.90 ± 0.05	1.22 ± 0.09	0.85 ± 0.06
Styrene 7,8-oxide[b]	4.80 ± 0.33	5.31 ± 0.33	3.91 ± 0.28
Bromosulfophthalein	Below the limits of detection		
Cumene hydroperoxide[d]	Below the limits of detection		

[a] GST activity towards various substrates was determined as described in *Materials and Methods*. Each value represents the mean ± SD of at least three samples assayed in duplicate.
[b] nmol/min/mg.
[c] pmol/min/mg.
[d] Glutathione peroxidase activity determined at 25°C.

Raza et al (1991) J Invest Dermatol 96:463-467

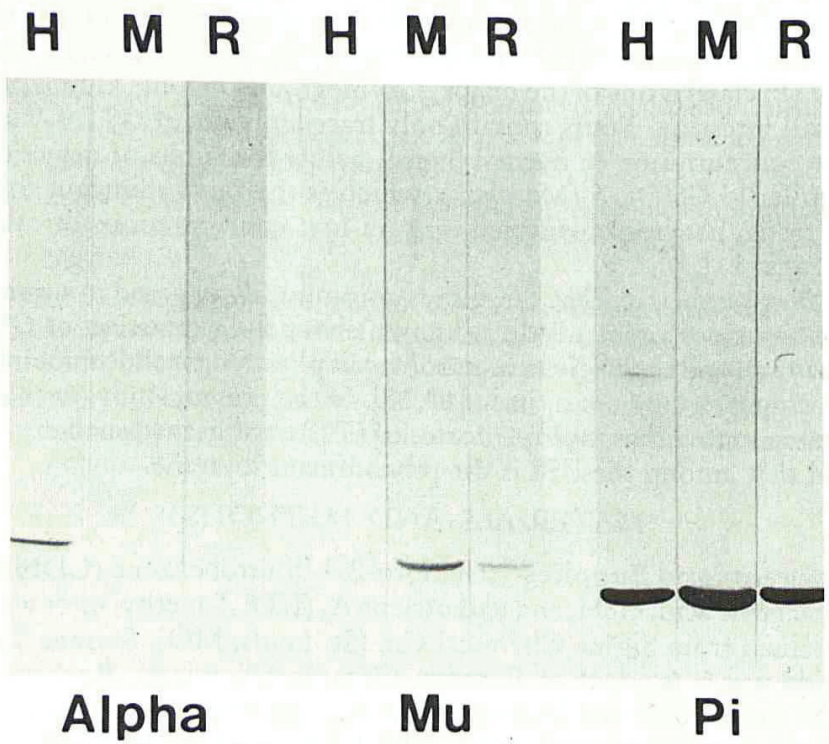
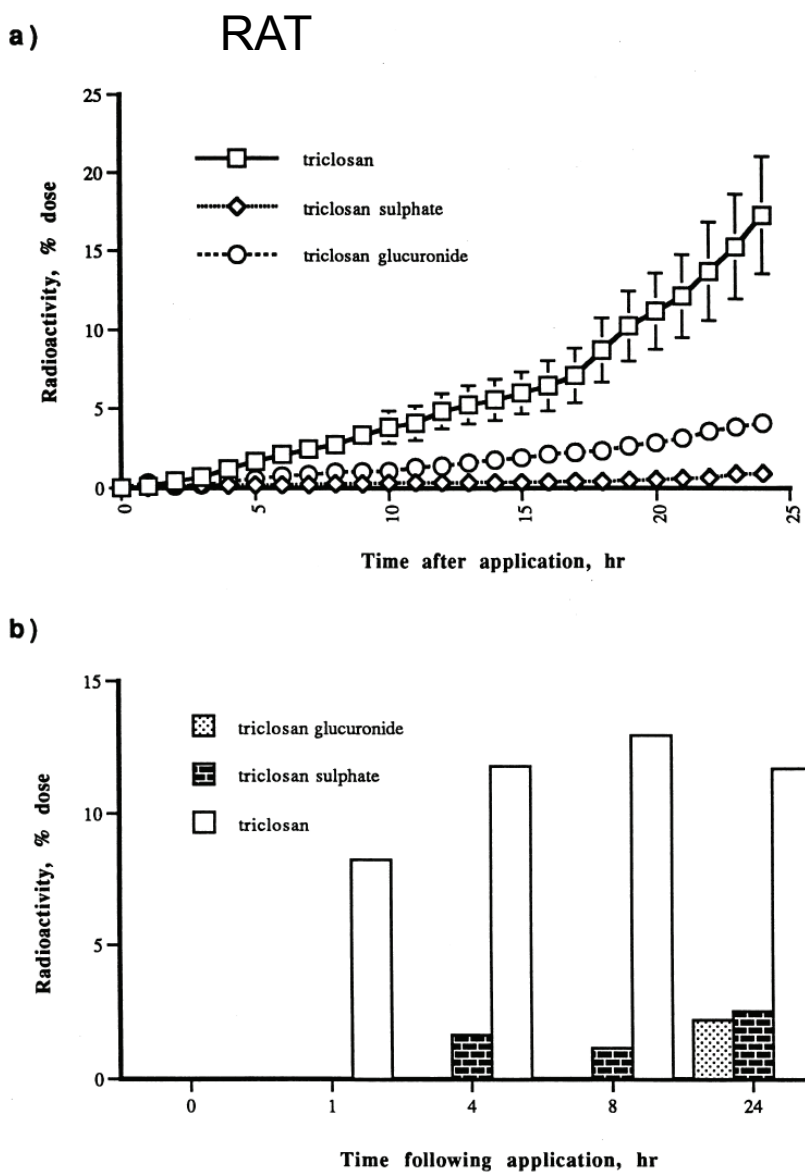
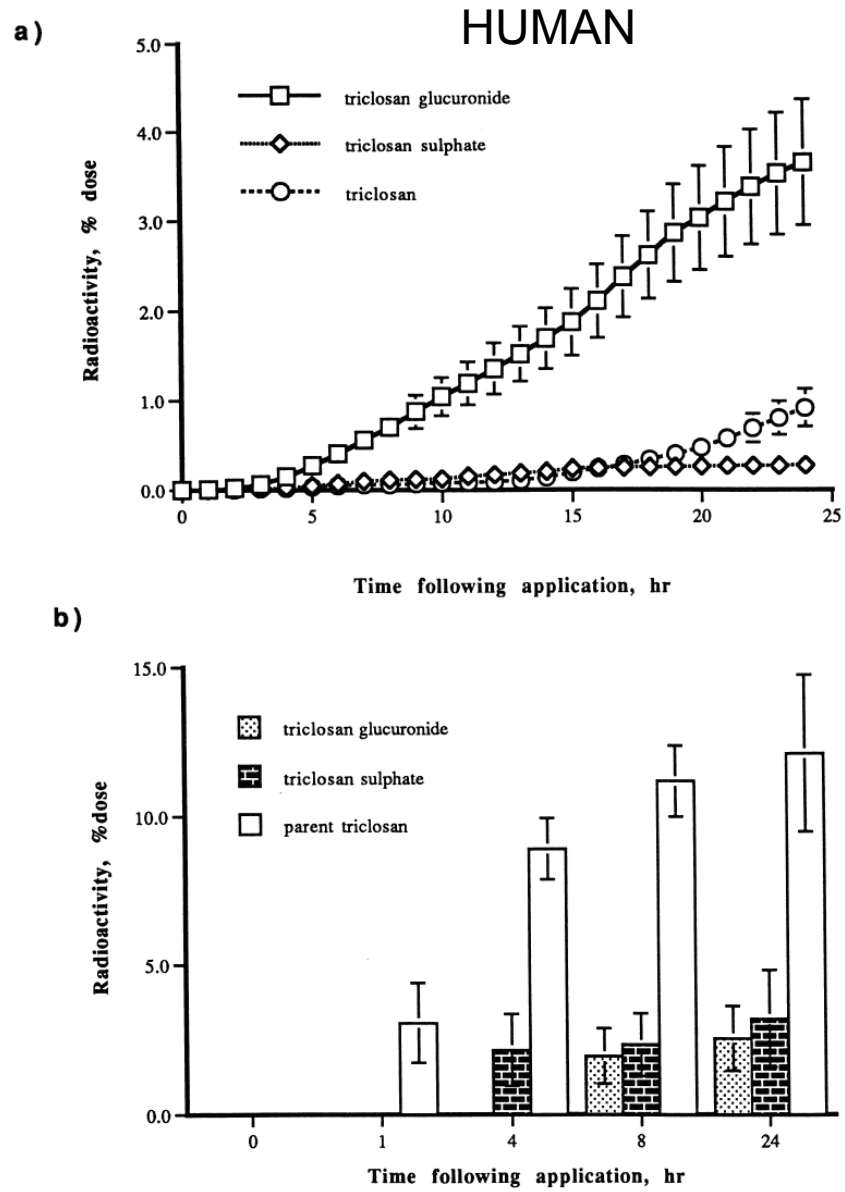


Figure 1. Western blot analysis of human and rodent skin cytosol. Skin cytosolic protein (70 μg) from human (H), mouse (M), and rat (R) was subjected to 12% SDS-PAGE and transferred onto nitrocellulose membrane by Western blotting as described in *Materials and Methods*. The membrane was probed with human polyclonal rabbit antibodies to GST (1 : 1000 dilution) and color was visualized by alkaline-phosphatase – conjugated secondary antibody.

GST isoforms expressed in skin cytosol – species differences

Raza et al (1991) J Invest Dermatol 96:463-467





Health Protection Agency

Skin Metabolism Meeting CR Europe
20th May 2011

Newcastle University

Species differences

- GST activity in human skin cytosol generally lower than rat or mouse
- GST activities in human skin subcellular fractions five fold higher than rat or minipig skin
- Esterase activity towards ethyl nicotinate high in rodent spp, much lower in human skin

Summary

- Important advances in cutaneous metabolism.
  - Expression, enzyme activity in skin and keratinocytes of full range of Phase I and II activities
  - products of metabolism during absorption in vivo/ex vivo skin for some Phase I and II activities
  - Localisation in (basal) epidermis, appendages
  - Involvement of AhR
  - involvement in differentiation

The future

- Development of standard protocols for "qualitative" and quantitative predictions of metabolic activity in skin to replace the local lymph node assay
- A better understanding of the relationship between metabolism of endogenous and xenobiotic substrates
- Novel in vivo/in vitro approaches to skin absorption and metabolism

Acknowledgements

- Clive Roper
- Chris Jewell
- Tim Moss
- David Lockley
- Matt Traynor
- All contributors to the field of cutaneous metabolism research past and present