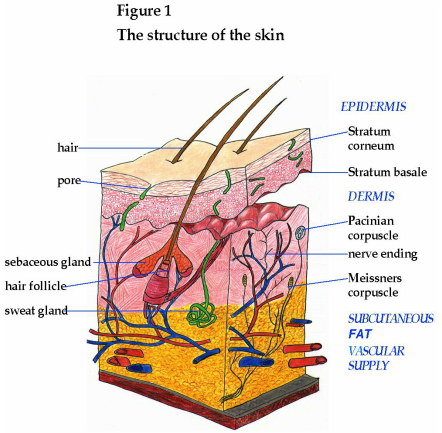
Human skin metabolism
Past present and future



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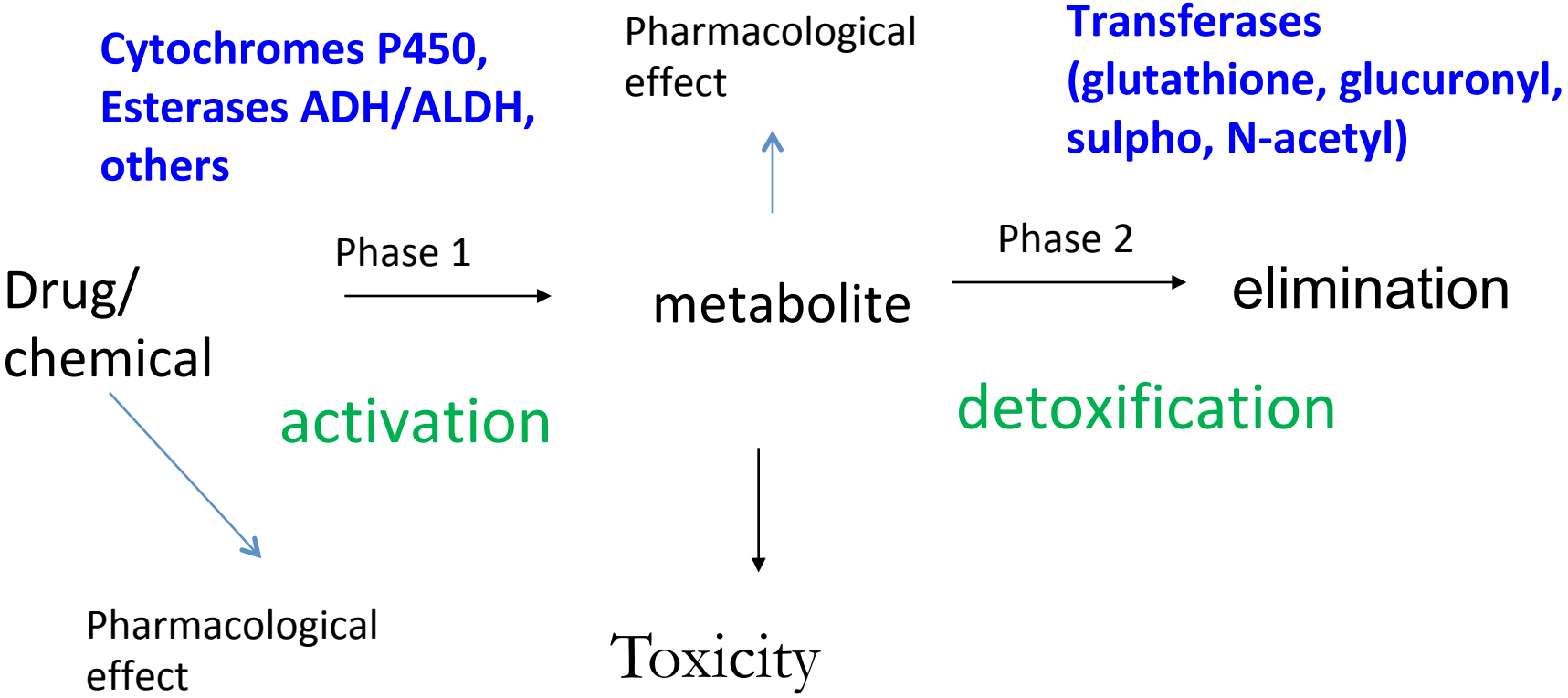
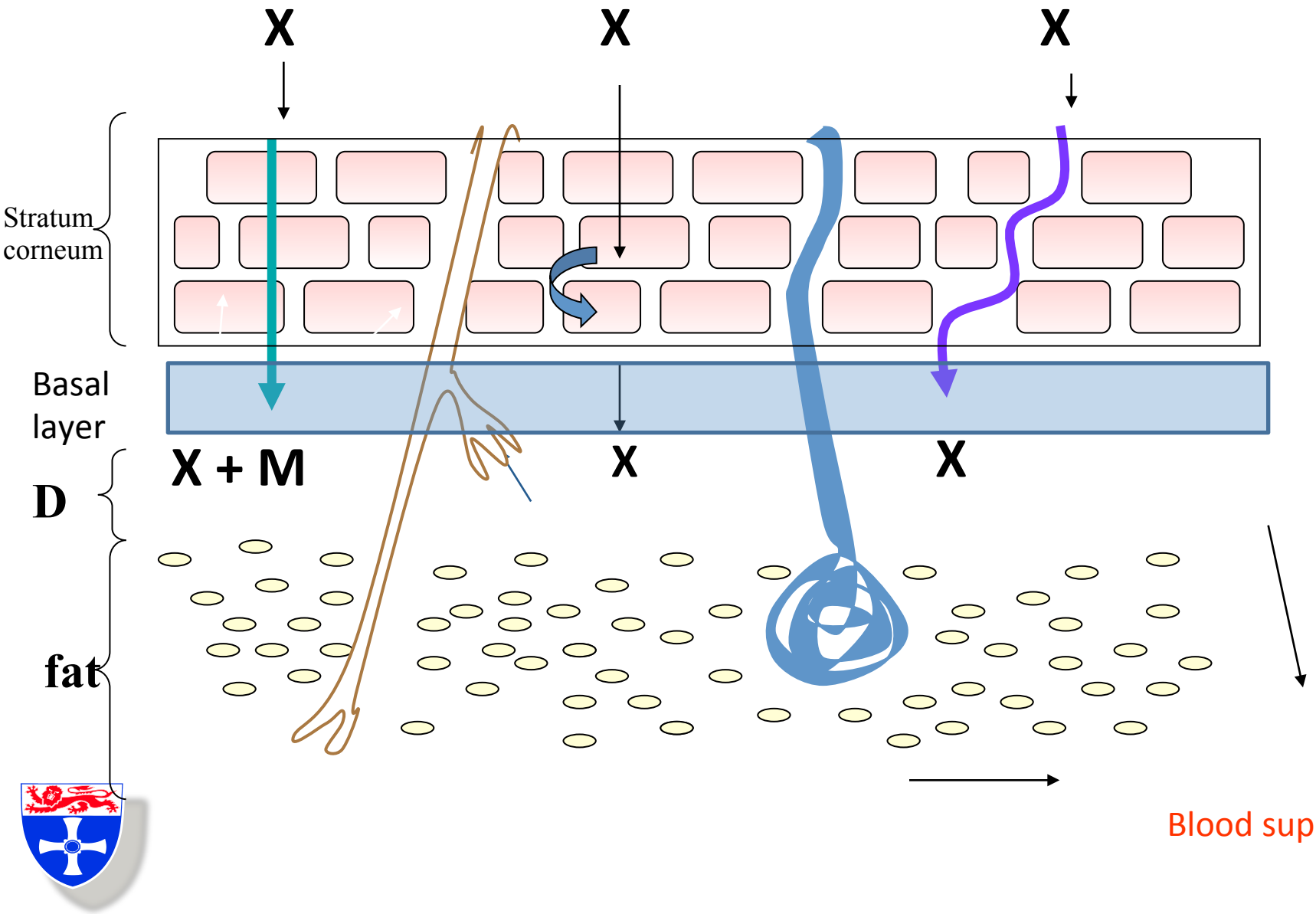
Metabolism of xenobiotics



Absorption and metabolism through the skin



Metabolism in the skin

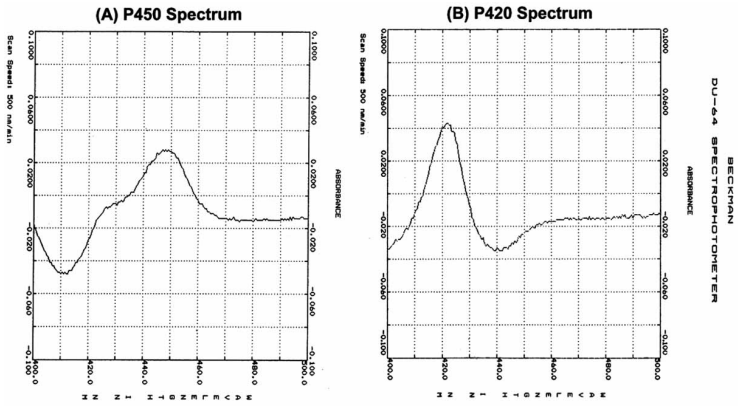
- *in vivo* difficult to distinguish from liver metabolism.
- Conversion may be low (CYP p450).
- P450 activity important for local toxicity eg DNA adducts formation of diol epoxides- toxicity local binding, sensitisation, irritation
- or may affect the bioavailability of the parent absorbed material- First pass deactivation eg conjugation, hydrolysis,
- local metabolism - therapeutics eg local release of steroids from ester prodrugs

Defining enzymes present in skin-

- Functional measures – specific substrates-
- Protein expression- western, IHC
- mRNA Gene array

- subcellular fractions single pathway -loose cellular localisation and may dilute activity- Actually higher levels in cells
- Localisation to basal cells and hair follicles plus high levels in sebaceous and sweat glands.
- Fresh viable skin in short term culture/ static cells
- Studies in keratinocytes fresh, in culture and cell lines
- Skin equivalents- in house or commercial

early understanding of dermal metabolism



- cytochromes p450 low in skin- CO binding protein
  Cyp p450/p448--- p420

- specific functional
- profile – differs from liver

- Only v low activity enough= toxic metabolite

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.

CYP activity in human skin

- Our data – CYP activity detectable but low, interindividual variation need absolutely fresh tissue. Activity lost on freezing.
- Need sensitive assay for product
- Aldrin – gc/ecd

Fluorescent substrates Benzopyrene

–Testosterone- LC-MS

Recent groups Skin or LSE models low, variable undetectable but inducible

Immuno assay, m RNA

fresh skin still essential

Not all oxidative activity CO inhibited- myeloperoxidase

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 M KCl–phosphate buffer, pH 7·5.

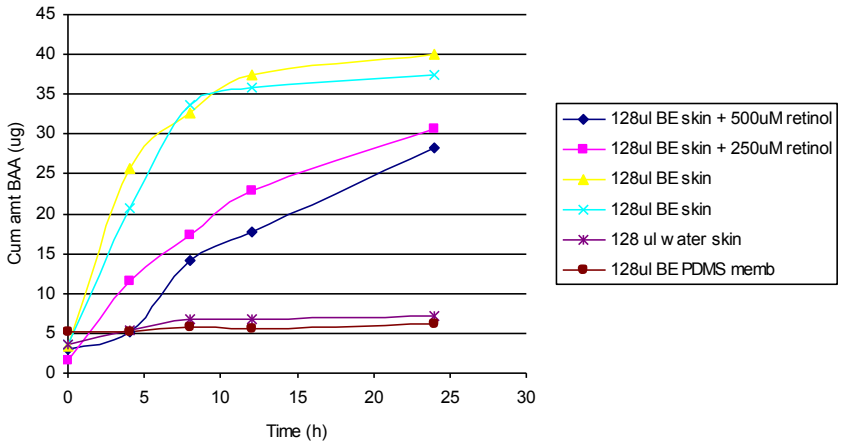
Rettie AE, Williams FM, Rawlins MD (1986) Xenobiotica, 16: 205-211

Phase 1 enzymes in skin substrates

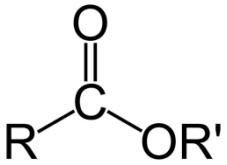
- Cytochrome p450
  - 1A1 Benzopyrene
  - 1A1 – 2B Resorufin analogues
  - 2B Aldrin
  - 1A1 +Ethoxy coumarin
  - 2 C9 +7 methoxy trifluoromethyl coumarin (MFC)
  - 3A + Testosterone
  - 7 benzyloxyquinoline
  - naphthalene
- FMO 1 and 3
- myeloperoxidases
- carboxylesterases
  - Paraben esters
  - Fluazifop butyl
  - Umbelliferyl esters
  - Carbaryl
  - Organophosphates
- Cholinesterases
- Alcohol dehydrogenases
  - Alcohols
  - Glycol ethers

alcohol dehydrogenases

- Alcohol dehydrogenases- activity in intact skin and subcellular fractions
- Activity greater than CYPs
- •Protein expression (ADH1, 2 and 3; ALDH 1 ADH profile detected in skin by Western blotting differs from liver
- •Histochemical localisation to epidermis and appendages. Little ADH2 detected.



Carboxylesterases in skin



- Carboxylesterases hydrolyse esters in the skin.
- mainly hCE1 (liver) and hCE2 in extrahepatic tissue
  - Skin like small intestine.
  - Microsomal and cytosolic
    - substrate specificity
    - Differing inhibitor sensitivity

Marker esters

P nitro phenyl acetate

Phenyl valerate

Phenyl acetate

Methylumbelliferyl

Acetate

Carbaryl

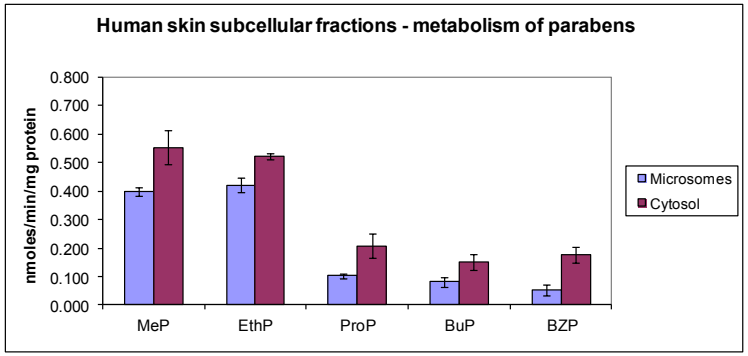
Fluazifop butyl

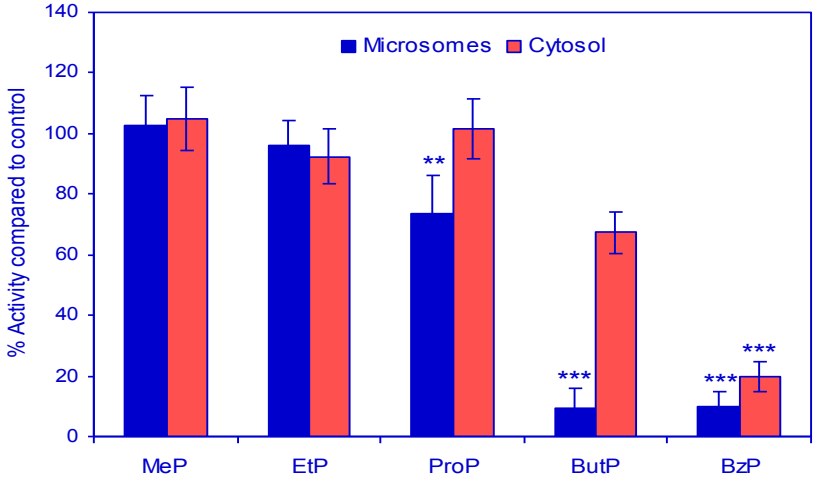
Procaine

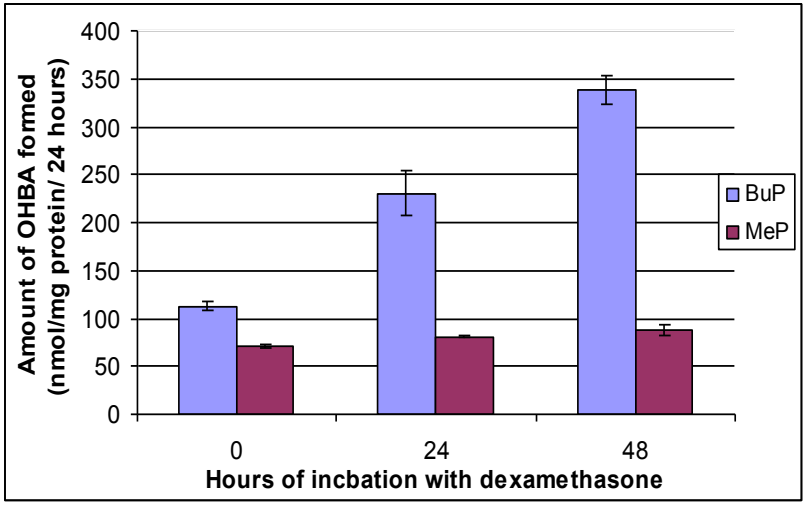
Paraben esters

Carboxylesterases in skin

- Methyl paraben to butyl / benzyl paraben
- Can be upregulated by environment (chemicals UV) steroids
- Inhibition of hydrolysis of parabens by loperamide (hCE2 inhibitor)







Jewell et al, Williams et al 2013

Phase II metabolism in skin

Generally higher compared to CYP than liver

Extrahepatic tissue profile

balance of pathways- detoxication

- Glutathione S transferases
  - DNCB GSTP
  - Cofactor limiting
- N acetyl transferases

  NAT-1 and NAT-2
  - Pamino benzoic acid NAT1
  - paraphenylenediamine

Phase II metabolism in skin

Sulphotransferases

- SULT1A1-E
- SULT 2B1 – steroids-cholesterol

Glucuronyltransferases

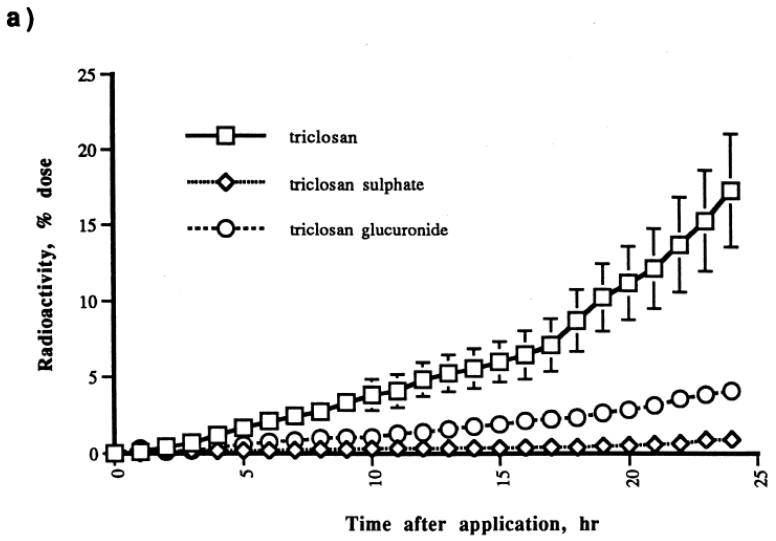
- UGT1
- UGT2

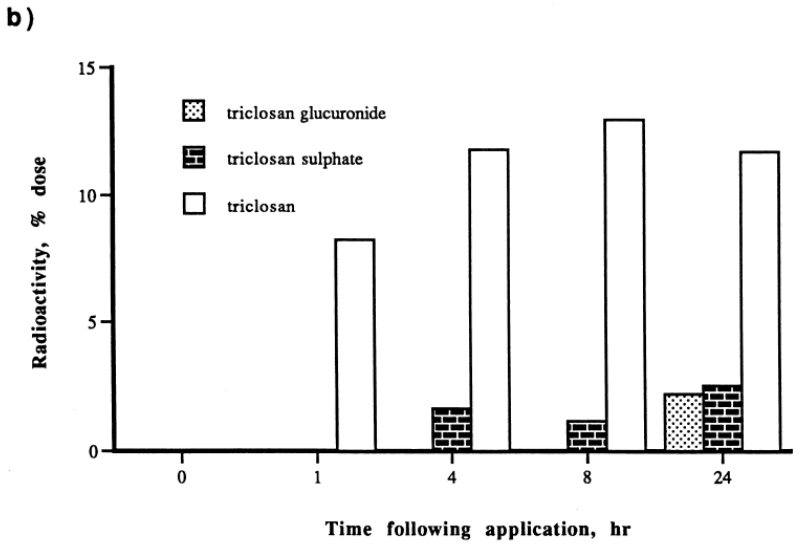
- Phenols P nitrophenol
- Dopamine
- Triclosan
- Minoxidil
- Bilirubin
- SULT2B1b cholesterol
- Dopamine
- 5HT
- 4 Methylumbelliferone UGT1
- Pnitro phenol
- Bilirubin
- deoxycholic acid

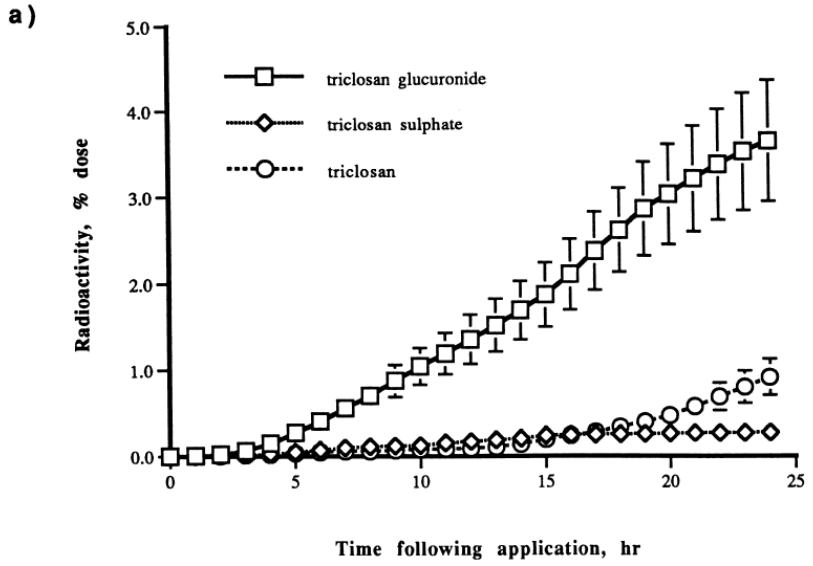
Triclosan

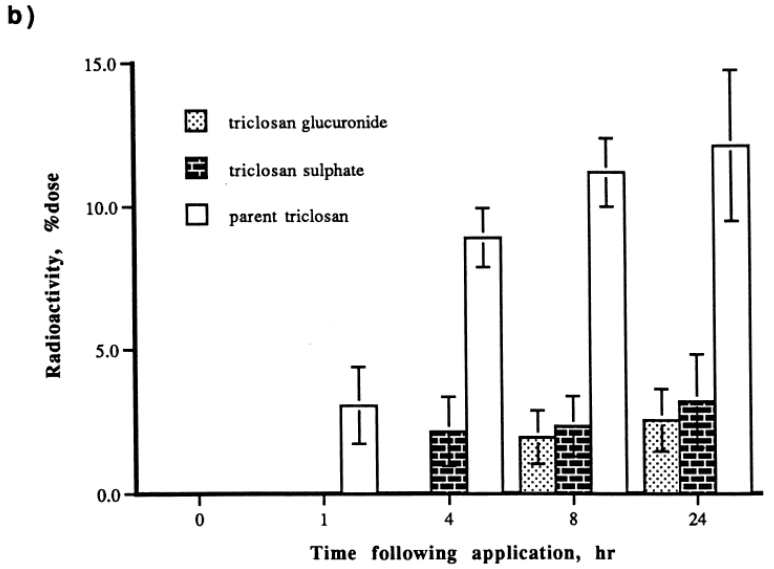
RAT

HUMAN

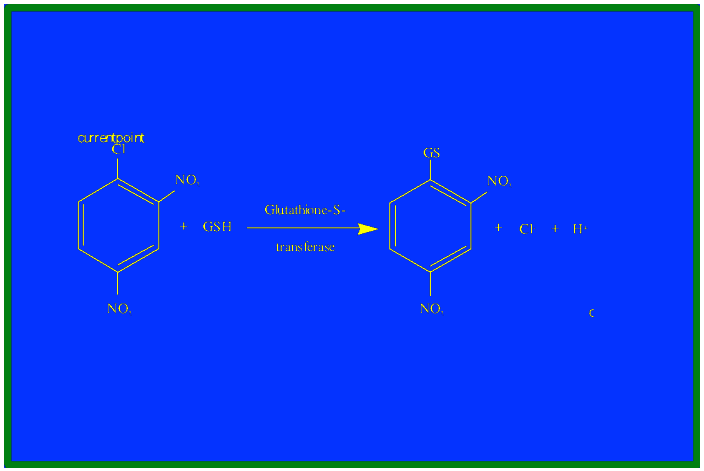






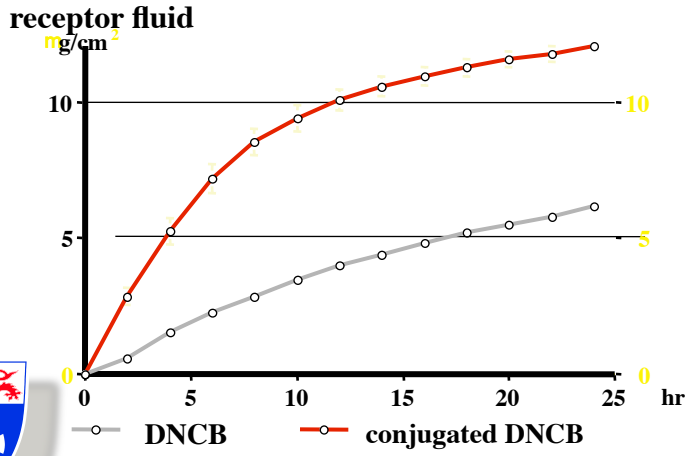


Glutathione transferases

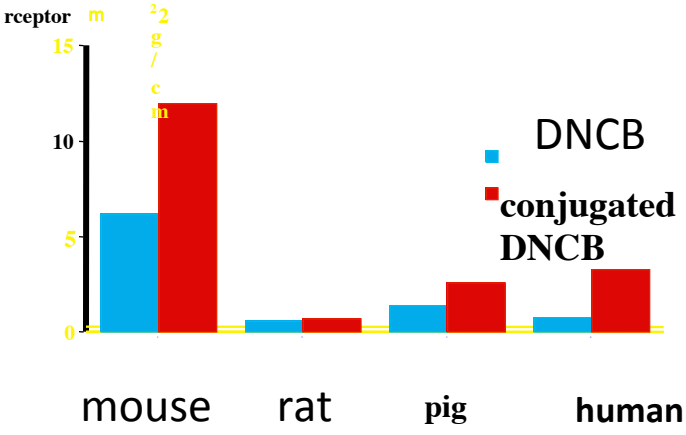


Mainly GSTP (DNCB substrate)
CDNB less specific
Glutathione may be limiting
- sensitisation

Metabolism of DNCB applied to skin in flow through cell



Absorption of DNCB through skin in the flow through cell in 24 hr



Jewell et al

Current approaches

- Emphasis on evaluation of models-
- Define activity, protein expression and RNA profiles
- Cosmetics Europe paper – good summary
- Points:
- Issues with frozen skin,
- Marker substrates
- Induction and knock down

Concerns

- Absorption versus metabolism in “Short term culture” – conditions do not reflect those in vivo particularly regarding time;
- Need not only define marker substrates but chemicals of concern local and systemic toxicity
- Relate to other routes small intestine and liver

Future:

- Important to relate to in vivo studies – generate data for PBPK modelling
- Absorption -Rate of diffusion through skin versus access to metabolising enzymes
- Vmax of enzyme- potential for saturation at high topical application
- Uptake and efflux from cells- transporters?
- Limitation of cofactor availability eg glutathione
- Oral dermal extrapolation- influence of metabolism differences

Acknowledgements

- Everyone !
- Simon Wilkinson
- My research groups
- Collaborators
- Funding

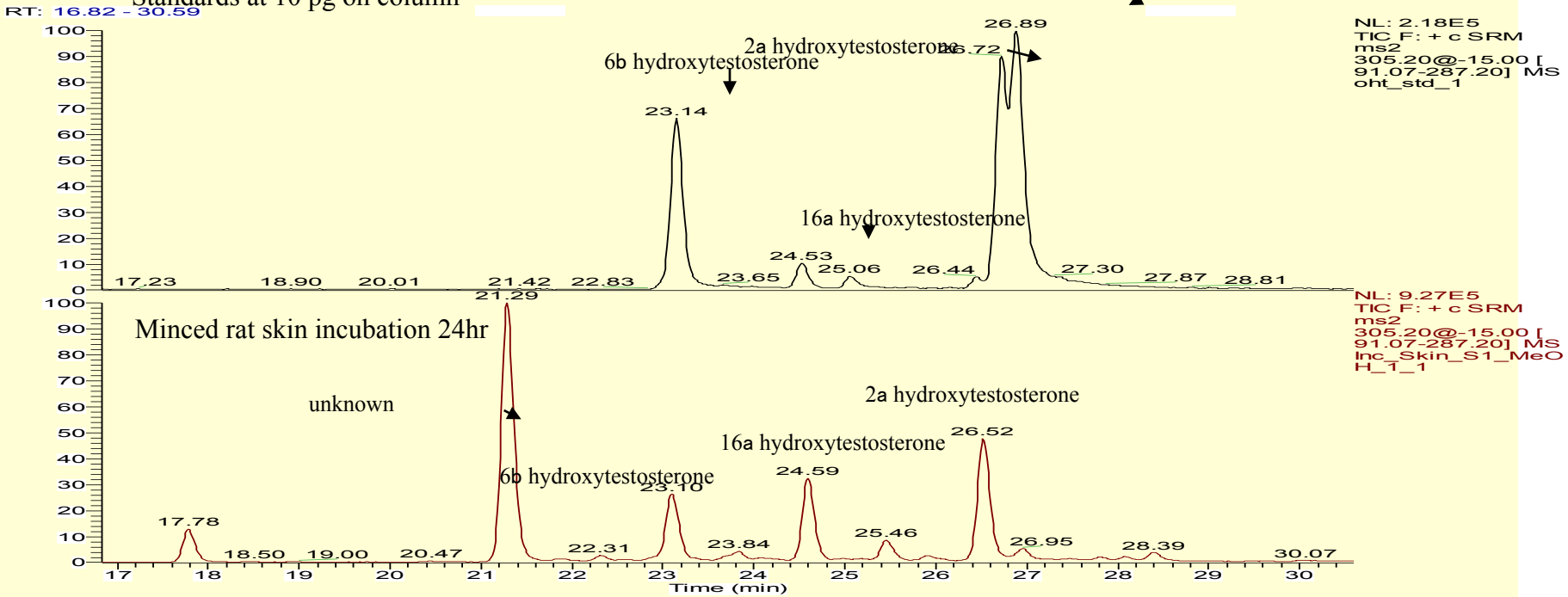
Hydrolytic activity in rat skin cf lung and liver microsomes/cytosol

Vmax	Liver	Lung	Skin
Fluazifop Butyl	m 6.2	m 0.4	m 0.02
(mmol/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

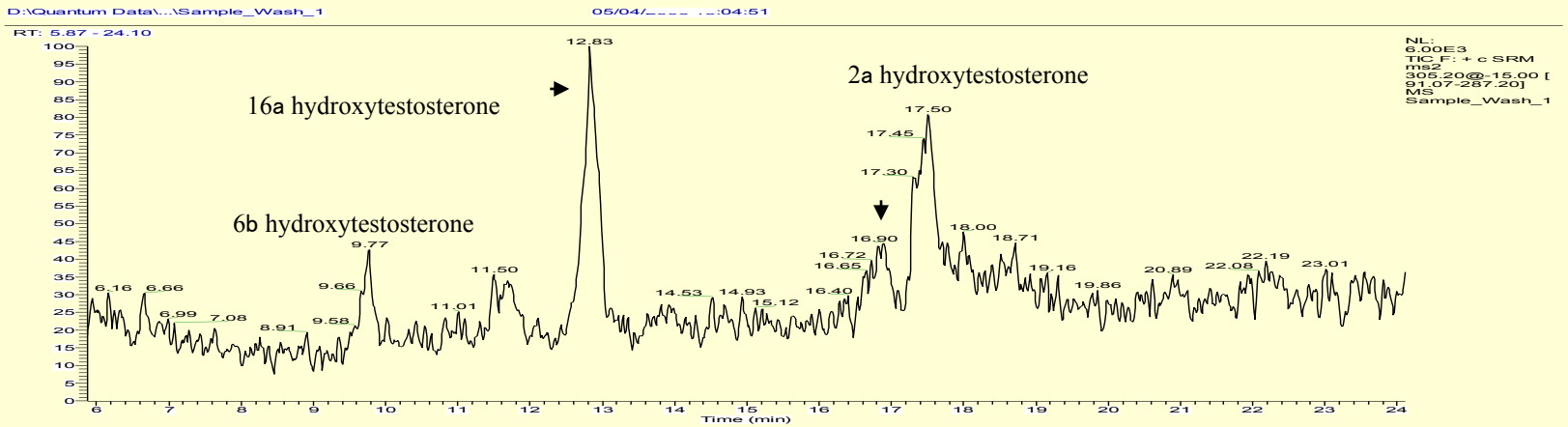
Phenylacetate activity detected in all tissues
Hydrolysis of FB was inhibited by paraoxon and bisnitrophenol phosphate
McCracken et al (1993) Biochem Pharmacol 45: 31-36

Results *Testosterone metabolism during dermal absorption*

Standards at 10 pg on column

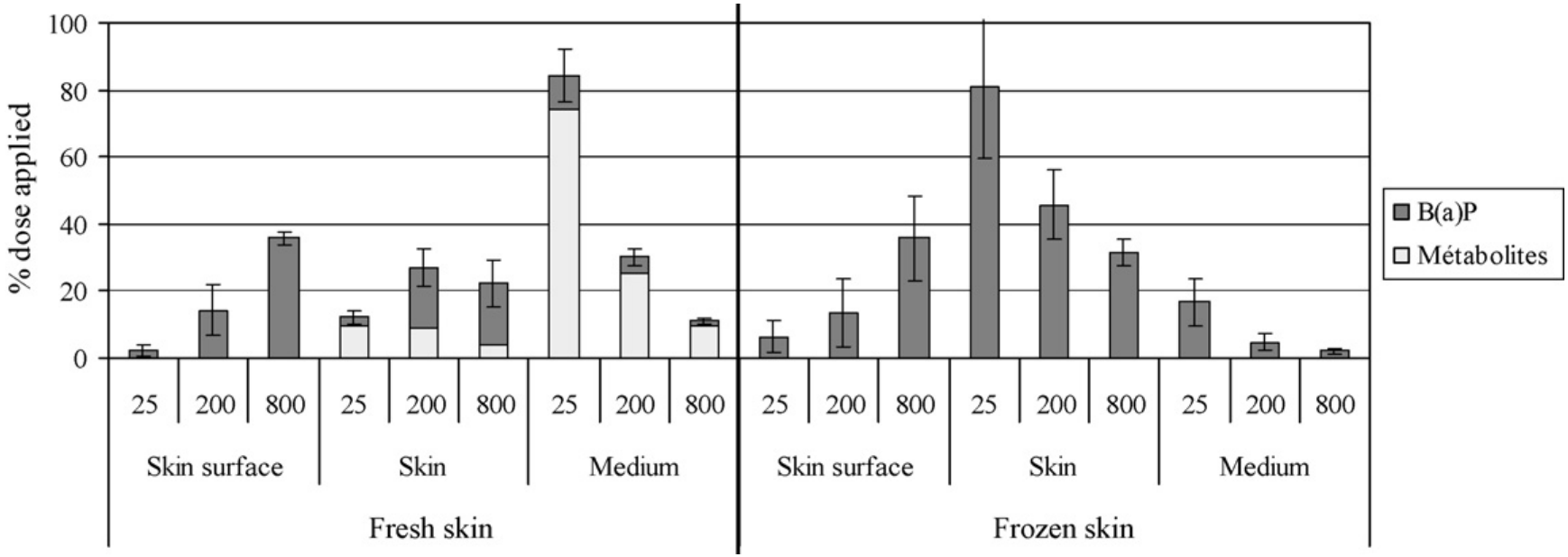


Receptor fluid from whole rat skin 8 hour



quantitative detection limit at about 1 pg on column, - 100fg/ul

Lack of stability of CYPs
Benzo[a]pyrene in pig skin



Parent and metabolites after 72 h in organotypic culture
Jacques et al. (2010) Toxicology Letters *199 22–33*

Previously fresh Human skin blisters – benzoapyrene metabolism (Chapman et al)