(Special Overview)

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Metabolism and Toxicity of Chemical Substances

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INTRODUCTION

I joined the study of drug metabolism under the leadership of late Prof. Hisao Tsukamoto, Kyushu University about 30 years ago. During these years, the studies of drug metabolism have developed unexpectedly. Now, no pharmacist can understand the action and toxicity of drug without knowledge of metabolism.

Today, I want to review generally the situation of drug metabolism concerning with specially toxicity. We usually use the term drug metabolism but we are considering all the chemicals for not only drugs. At the present time, as shown in Table 1, there are a great many chemicals in our environment and we are utilizing many of them for drugs of human and animals, pesticides, food additives, etc. We can share in the benefit of pharmacological action of drugs or useful properties of chemicals and we wish to drive out the adverse effect from the chemicals including drugs. For this purpose, we have to understand the mechanisms how chemicals express their toxicity and how we will be able to avoid the toxic effect.

It seems in common sense that poisons and drugs are two entirely differnt things. But, it is not true in scientific sense. The primitive men must have recognized poison as material not good to eat. Nowadays, we can enjoy our daily life comfortable by the aid of a lot of chemical substances. Drugs can cure persons of diseases. Food additives can preserve foods and make the cost of foods cheap. Pesticides can kill noxious

Table 1. Chemical substances

	Benefit	Risk
Drugs for human	cure or protect diseases	adverse side effect
Drugs for animal		drug tolerant bacteria
Feed additives	growth promotion	residual toxicity
Pesticides	kill insect increase harvest	
Manure		
Food additives	preservation, etc.	toxicity
Chemicals in home life		carcinogenicity
Industrial chemicals	convenient life	mutagenenicity
		allergy, etc

insects and increase harvest. On the other hand, we have to suffer sometimes from the undesired adverse effects of chemicals.

Even a same compound, it could be drug or poison depending on dose. We can not qualitatively distinguish poison from drug. Even though water, it will be harmful if it will be given too much. Alcholic drink is called as the king of drugs, but also the mad water.

There is no difference between pharmacological effect and toxic effect from the standpoint of drug-living body interaction. If the beneficial effect or the adverse effect depends on mind of human. Therefore, I will talk about the metabolism of chemicals in general sense without the difference between drugs and toxic substances. That is why I entitled this speech as chemical substances.

At this time, I would like to consider "chemicals" which include all the chemical substances ingested into biological body, regardless of whether drugs for medicine or other useful or harmful substances. Even though the constituents of body and food which are essential tor life, if they will be exogeneously given too much, they must be considered as xenobiotics.

DYNAMICS OF CHEMICALS IN THE BODY

Fig. 1 shows the movement of chemicals in the body. The chemical substances ingested into body are absorbed in blood, distributed to tissues and organs, metabolized mainly in liver, and excreted out through urine, feces, expiration air, etc. The parent compound and/or metabolites reached the acting sites and expressed its action or toxicity. As shown in Fig. 2 the main stream of drug metabolism is the increae of polarity followed by the increase of lipid solubility for easier excretion through urine. This proceed in the direction of detoxication,

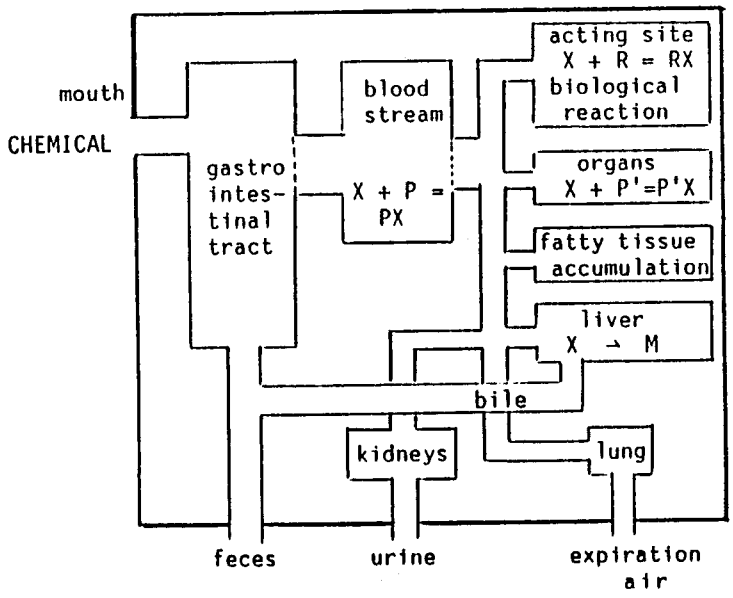


Fig. 1.

releasing foreign compound from the body. However, not all the metabolites are reduced their activity, but rather much more active metabolites are frequently produced. As I will review later, the metabolic acitivation is an important theme in toxicology. The metabolism holds a key to detoxication and activation of chemical substance in the body.

Concerning with absorption, it has been expected to develop the useful transdermal preparation of drugs. It is difficult to describe the real mechanism of absorption by this route, because there are many factors. We have observed that lecithin (PC) stimulated the absorption of indomethacin (IM) after topical application by determining the skin, blood and urine level of 14C-IM as shown in Table 2,3,4. The most acceleratve effect was found in IM gel ointment containing both benzylnicotinate (NB) and PC. It is expected to reduce the adverse effect of IM by reducing the dose which is necessary for the same pharmacological effect.

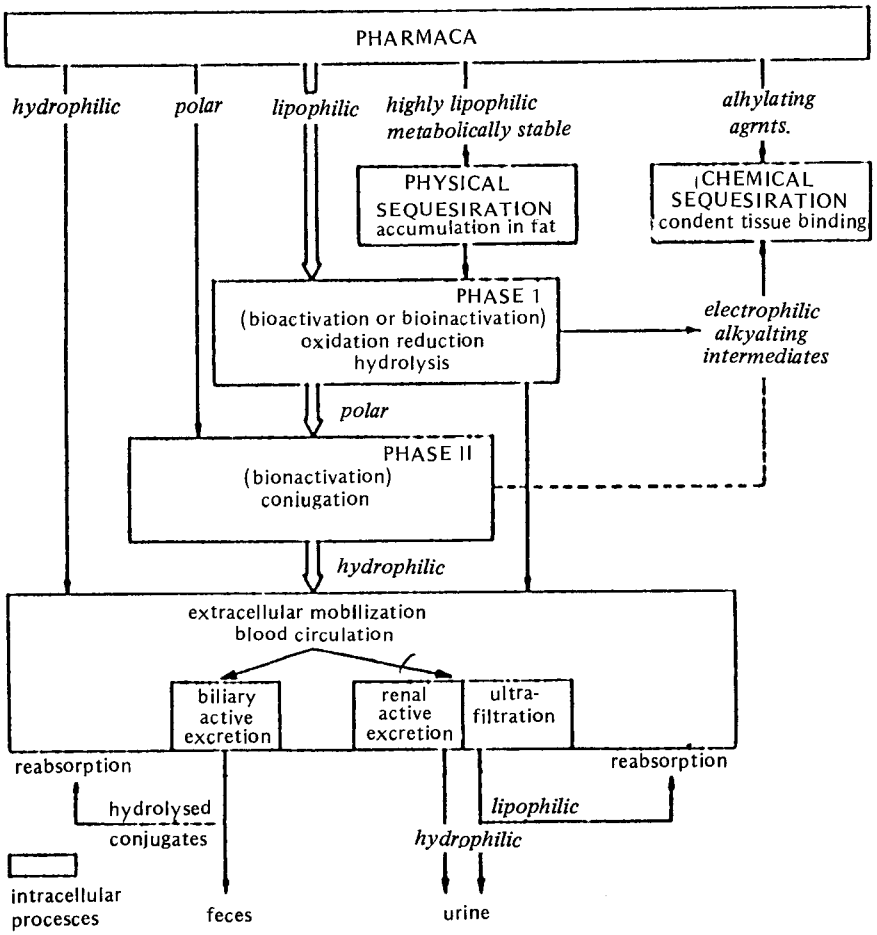


Fig. 2. Schematic representation of the most important aspects of pharmacon metabolism. The general trend is transformation of Hpophilic substances to more hydrophilic substances where toxicity is reduced and excretion via the urine is enhanced.

Table 2. Plasma AUC

	Drug	AUC (μgH/ml)	MRT (H)	VRT (H)
I	control	5.31	10.71	21.11
	+ NB	6.42	9.96	16.40
II	control	3.00	8.98	19.17
	+ PC	5.65	8.58	22.13
III	control	5.28	9.23	15.01
	+ NB + PC	16.83	7.80	19.90

Table 3. Absorption of 14-C

Drug	% of dose	
–	10.49 ± 1.68	
NB	16.60 ± 5.40	
PC	40.67 ± 6.29	$P < 0.01$
NB + PC	38.12 ± 4.37	$P < 0.01$

$$\text{absorption (\%)} = 1 - \left(\frac{\text{recovery 7hr after application}}{\text{recovery at 0 hr}} \right) \times 100$$

Table 4. Absoption of 14C-1M through the carrageenin induced rat back skin edema after topical application of 14C-1M + NG + PC Gel

	% of dose	
	Absoption	in Skin
Intact	12.67 ± 1.92	2.90 ± 0.21
Edema	19.32 ± 2.27*	4.16 ± 0.42*

N = 8–9, * : $P < 0.05$

OUTLINE OF METABOLISM OF CHEMICAL SUBSTANCES

First of all, I would like to review the general situation of drug metabolism with some consideration from historical point of view. Then, I will show the results of our experiments performed with many collaborators concerning with this theme.

The first investigation of drug metabolism was the biotransformation of benzoic acid to hippuric acid and these studies were performed by German chemists in the first half of the 19th century. Hippuric acid was also found in the urine of dog and rabbit received cinnamic acid or benzaldehyde. Thus, the glycine conjugation and oxidation were the first discovered metabolic process.

The major metabolic pathways known at the present time were successively discovered in the latter half of the century. They were etheral sulfate formation, ornithine conjugation, mercapturic acid formation, glucuronic acid conjugation and so on. However, the studies in these period had been mainly chemically carried out for the urinary metabolites from the animals received the chemicals. They were the fundamental studies in biochemistry and not a few of biochemical rules had been discovered. However, the enzymatic studies had to be waited for more than 50 years.

However, in earlier period, the drug metabolism had been understood as so called detoxication mechanisms. Neumeister published a book "Lehrbuch der physiologische Chemie" and opened a chapter as "Entgiftung", that is "detoxication" in 1895. He recognized the drug metabolism as a process of protecting mechanism. In 1921, Sherwin published a review article entitled "The Fate of Foreign Organic Compounds in the Animal Body" and described that the animal must excrete foreign compounds by degrading or any other process for protecting themselves.

The late Prof. R.T. Williams, London, one of the greates investigators of drug metabolism, published a famous book "Detoxication Mechanisms" in 1947. In his review article published in 1951, he described that the learning of metabolic fate was necessary to elucidate the mode of

drug action and to develop of new useful drugs and expected that the studies in drug metabolism could be rapidly developed by the aid of radioisotopes and the newly developed analytical tools.

After about 1950, the investigation of drug metabolism entered in a new phase and it has been very rapidly and widely developed as Dr.

Table 5. Microsomal oxidation of chemical substances

$A-H + NADPH + H^+ + O_2 = A-OH + NADP + H_2O$

monooxygenase (mixed function oxidase)

A–H → A–OH oxidation

NADPH → NADP dehydrogenation

NADPH → fp_2 (FAD, FMN) → cytochrome P–450 → O_2 (1)

NADH → fp_1 (FAD) → cytochrome b_5 → CSF → O_2 (2)

fp_2 : NADPH–cytochrome P-450 reductase

fp_1 : NADH-cytochrome b_5 reductase

CSF : cyan sensitive factor (non-heme)

(1) : oxygenation of chemicals

(2) : desaturation of fatty acids

Table 6. Enzymes participating in drug metabolism

Phase I Transformation	
Microsomal Cytochrome P-450	
Oxidation	Reduction
Alkyl side chain	Azo
Aromatic ring	Nitro
Alicyclic ring	Reductive dehalogenation
N-Dealkylation	N-Oxide
O-Dealkylation	Epoxide
N-Hydroxylation	
Deamination	
S and O exchange reaction	
Epoxidation	
Non-cytochrome P-450 Enzymes	
Oxidation	Reduction
Alcohol dehydrogenase	NADPH-cytocchrome c reductase
Aldehyde dehydrogenase	Xanthine oxidase
Aldehyde oxidase	Sulfoxide reductase
	Sulfone reductase
	Aldehyde reductase
Hydrolase	
Hydrolysis	
Esterase, Amidase, Glucuronidase, Arylsulfatase	
Epoxide hydrolase (Ms)	
Phase II Transformation	
Conjugation Enzymes	
Glucuronyltransferase	–OH, –COOH, $-NH_2$, etc.
Glutathione transferase	Epoxide, nitro, halogen, etc.
Sulfotransferase	Aromatic –OH, $-NH_2$, etc.
Acyltransferase	Aromatic $-NH_2$, etc.

Williams expected. It has become a fundamental field of biological sciences. The knowledge that the metasbolism is closely related with not only detoxication but also activation has been fixed by the work of Dr. B. B. Brodie NIH, Bethesda, U.S.A., a great pioneer in this field.

The most important discovery by Dr. Brodie's group was that the drug oxidation was performed by an enzymatic system called as drug metabolizing enzymes in hepatic microsomal fraction as shown in Table 5. This enzyme requires reductive pyridine nucleotide phophate, NADPH, and molecular oxygen. Unique characters of drug metabolizing enzyme had been pointed out. They were broad substrate specificity but ability to distinguish foreign compounds from native components.

On the other hand, Dr. Sato and Dr. Ohmura found a new cytochrome called as P-450 in microsomes, which showed the maximal absorption peak at 450 nm after binding with carbon monooxide in reductive state in the studies of electron transport mechanisms in hepatic microsomes. At first, these two findings were not directly connected. But, several years later, it was made clear that cytochrome P-450 played a main role in drug oxidation or drug metabolism. Since that, a great many studies have been performed from various points of view.

The oxidation by cytochrome P-450, an mixed function oxidase, has been elucidated as quite popular mechanism. A great many biologcial constituents are oxidized by this enzyme in the process such as fatty acid oxidation, steroid oxidation, etc. Cytochrome P-450 was found not only in liver but also in other organs such as kidneys, lung, adrenals, etc. and not only in mammals but also various biological bodies including microorganisms and cytosol. The attention is recently given to peroxisomes especially for alkyl chain oxidation.

At the present time, the metabolic pathways are divided into two main categories: the first phase and the second phase transformation as shown in Table 6. The first phase reaction consists of oxidation, reduction and hydrolysis. These reactions produce the new functional groups such as hydroxyl group, amino group, etc. The functional groups in the parent compounds or metabolites are conjugated with various biological constituents such as glucuronic acid, sulfuric acid, amino acids, glutathione, etc. This phase of reaction is a synthetic process.

Each transformation process is catalyzed by enzymes; cytochrome P-450 and other many enzymes. There is no specific enzyme acting for only xenobiotics. This Table shows the enzymes participating in drug metabolism and catalyzes various types of biotransformation and it is not only oxidation but also reduction. On the other hand, the oxidation is not catalyzed by only cytochrome P-450 but also by dehydrogenases, etc. Reduction and hydrolysis are also common pathways and also concerning with detoxication and activation.

The conjugation is also mainly enzymatically performed. Uridine diphosphate glucosiduronic acid (UDPGA) formed from glucose-phosphate by two steps gives glucuronic acid moiety to aglycone possessing alcoholic or phenol hydroxyl group, carboxyl group, amino group or sulfhydryl group by the aid of an enzyme, UDP-glucuronyltransferase. Glutathione is conjugated non-enzymatically but mainly through by enzyme. Conjugation had been considered as just detoxication but recently recognized as activation step, too.

The metabolic pathway is not limited to only one for one compound. One compound can be consequently attacked by various enzymes and the fate is complicated. Furthermore, the metabolism is affected quantitatively and qualitatively depending on the conditions of animal themselves and the external conditions where the animals are. Therefore, it is very difficult to predict the fate of a new substance and it is necessary to examine actual pathways in each case.

It was one of questions since earlier state of investigation whether cytochrome P-450 has only one or multiple forms. The purification of cytochrome P-450 has been succeeded after the introduction of glycerol as stabilizer, because this cytochrome is bound closely with membrane and labile for various treatments. At the present time, as shown in Table 7 at least about 30 kinds of cytochrome P-450 have been purified from liver and other organs of rats and rabbits pretreated with various chemicals as inducer.

FACTORS AFFECTING DRUG METABOLISM

Drug metabolism is affected by a lot of both endogenous and exogenous factors such as animal species, age, sex, genetic factors or environmental factors, etc.

Estimation of Drug Metabolizing Activity of Human

The species difference makes the estimation of drug metabolism in human difficult. On the process of new drug development, the investigation of metabolic pattern is an important problem in the first phase clinical test. We have to

Table 7. Multiple froms of cytochrome P-450

Inducer	Animal	Form of P-450	Specific substrate
Phenobarbital	Rabbit	P-450$_1$, LM$_2$	Benzphetamine (N-deMe)
	Rat	P-450$_b$, PB$_1$	
	Guinea pig		
3-Methylcholathrene	Rabbit	P-448$_1$, LM$_4$	Acetanilide (4–OH)
β-Naphthoflavone	Rat	P-450$_c$, MC–1	BaP, 7-EtO-coumarin (O-deEt)
	Mouse	P$_1$-450, P-448	BaP, Acetanilide
TCDD	Rabbit (neonatal)	From 6	BaP
Isosafrole	Rabbit	LM$_6$	BaP
	Rat	P-448 (high spin)	Estradiol-11β (2–OH)
		P-450$_d$	
PCB	Rat	P-448 II-a (high spin)	N-hydroxylation
		P-450$_b$, P-450$_c$	
Ethanol	Rabbit	LM$_{3a}$	Aniline, Ethanol
	Rat		
PCN	Rat	P-III, PB/PCN-E	Androstenedione (6–OH)
Clofibrate	Rat	Fr. 1	Lauric acid (w-OH)

TCDD : 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin
PCB : polychlorinated biphenyls
PCN : pregnenolone 16α-carbonitrile
BaP : benzo (a) pyrene
4–OH : hydroxylation at 4-position
deMe : demethylation
deEt : deethylation

know the total fate of drug from both qualitative and quantitative aspects. Table 8 shows the similarity of metabolic pattern of 22 drugs sampled at random in monkey, dog or rat with those in human. The points of similarity are high for between human and monkey, but low for between human and rat.

Table 8. Similarity of metabolic patients between human and animals

Animal drug	Points for similarity					
	Monkey		Dog		Rat	
	E.R.	M.P.	E.R.	M.P.	E.R.	M.P.
1		3				
2					2	0
3	2	2			1	0
4	3	3	1	1	1	0
5	3		0		1	
6		3				0
7		3				
8			2	2	1	2
9	2	0	0	0	0	0
10	1	0	0	2	0	0
11		1		1		1
12	1	0	3	2	2	2
13	1	2	2	2	2	2
14		2		1		1
15			2	0	1	0
16				3		3
17	3	2	0	0	0	1
18			2	1	2	2
19			1	3	2	3
20	3		2	0	0	0
21	2		1			
22			1	0	0	0
Point	Percentage					
3	30.0	33.3	7.1	13.3	0	11.8
2	40.0	33.3	35.7	26.7	42.9	11.8
1	30.0	5.3	28.6	33.3	28.6	17.6
0	0	25.3	28.6	26.7	28.6	47.1
Mean point	2.0	1.75	1.21	1.2	1.0	0.94

Point 3 : Highly similar
Point 2 : Moderately similar
Point 1 : Something silimar
Point 0 : Quite different
Mean point : Sum of point/Number of drugs
E. R. : excretion rate, M. P. : metabolic pathway

In order to make a dose plan for the most effective and safe application, it should be expected to know the metabolic activity of individual patient. For this purpose, we tried the application of two chemicals, one is benzydamine (BZY) and another is trimethadione (TMO). For avoiding pain from patients, it should be expected to give drug as small dose as possible and to take samples as few as possible. As shown in Fig. 3,4, it is possible to determine BZY and its metabolites, N-oxide and demethylated derivatives, at very low level by means of fluorometry in the 2 hr-serum and 4 and 8-hr urine samples from healthy volunteers and patients received a tablet of 25 mg. The excretion amounts of BZY and BZY N-oxide are higher in volunteers than in patients as shown Table 9. It might be possible to use the BZY and its metabolite level in serum and urine as an index of liver capacity of metabolism. However, the information obtained from this method is limited to estimate the total excretion pattern resulted from absorption and metabolism and it migh pass over the samll change of metabolic activity because of large substitution capacity of liver against too small dose of durg.

On the other had, TMO shown in Fig. 5 is rapidly absorbed from gastrointestinal tract and extensively N-demethylated to DMO. Neither TMO nor DMO is bound to plasma protein or any other macromolecules in biological materials. No major biliary excretion of this drug has been reported. The plasma disappearance of TMO follows first-order kinetics according to a simple one-compartment model system. Table 10 shows that the pretreatment of rats with carbon tetrachloride, *d*-galac-

tosamine and-naphthylisothiocyanate, prolonged $T_{1/2}$ and Tmax of TMO, reduced the Km value and increased AUC. These results suggest that plasma levels of TMO and DMO might be useful as an index of drug metabolizing capacity in animal and man. This work was mainly done by Prof. Kuroiwa's group, Showa University.

Induction and Inhibition of Drug Metabolizing Activity

The acitivity of drug metabolism is increased or reduced by a lot of chemicals. The induction or the accerelation of enzyme synthesis is very popular and important phenomena for cyto-

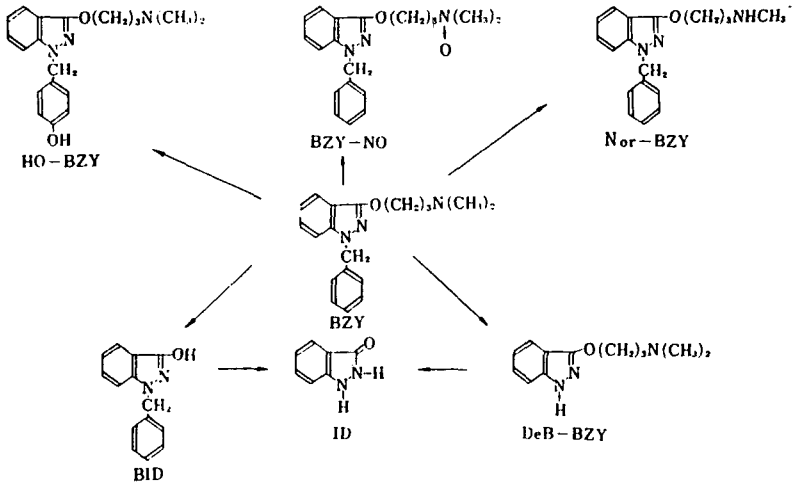


Fig. 3. Metabolic Pathways of benzydamine.

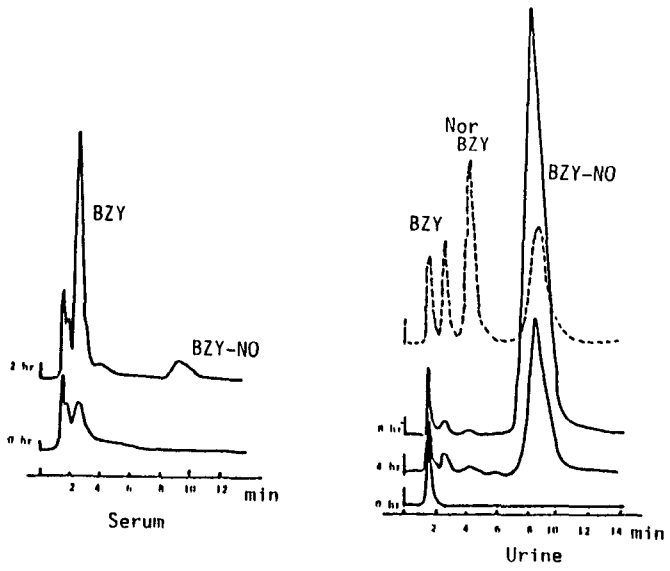
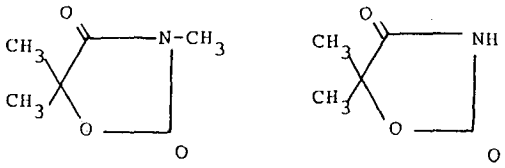


Fig. 4. HPLC pattern of serum and urine after BZY dosing.

Table 9. Comparison of excretion rates of BZY-NO and BZY in health volunteers and patients

BZY-NO	4 hr	8 hr
Volunteers	4.36 ± 0.65 (11)	8.68 ± 1.46 (11)
Patients	2.12 ± 0.44* (8)	4.44 ± 0.71* (8)
BZY	**4 hr**	**8 hr**
Volunteers	0.62 ± 0.20 (10)	1.36 ± 0.39 (10)
Patients	0.22 ± 0.04 (8)	0.51 ± 0.10* (8)

Values are mean and standard error of percentage of metabolites excreted after receiving of one tablet of 25 mg benzydamine hydrochloride.



Trimethadion (TMO) DMO

Fig. 5.

chrome P-450 and other enzymes. Phenobarbital (PB) and 3-methylcholanthrene (MC) are typical inducers of cytochrome P-450 but their inducing patterns are different each other. The chemicals possessing the activity of induction are mainly divided into two types, PB and MC type. The existence of multiple forms of P-450 was estimated by these difference. Many forms of P-450 have been purified from the animals pretreated with various chemicals.

PCBs, polychlorinated biphenyls have been considered as causal substance for Yusho disease outbroken in Kyushu area, Japan, in 1968 and Yu-Cheng in Taiwan in 1979. This chemical is also a world wide pollutant in the environment and foods. PCBs, a group of chemicals consisting of complex mixtures of chlorinated biphenyls, is a strong inducer of cytochrome P -450. There are 210 kinds of PCBs according to

Table 10. Pharmacokinetic parameter following the administration of trinethadione (100mg/kg)

Drug	C_o (μg/ml)	$T_{1/2}$ (h)	V_d (l)	K_{el} (l/h)	AUC (μg/ml/h)	C_{max} (μg/ml)	T_{max} (h)	K_m (l/h)
TMO								
Control p.o.	177	2.2	0.076	0.438	339	98.1	0.6	0.419
i. v.	136	2.5	0.094	0.415	362	–	–	0.425
CCl_4	134	12.3	0.100	0.064	2,104	113.0	1.5	0.068
ANIT	96	54.6	0.156	0.022	7,110	92.8	2.5	0.054
Galactosamine	100	39.6	0.136	0.052	4,307	101.4	0.8	0.112
DMO								
Control p.o.	179	39.4	0.073	0.019	8,120	162.8	8	
i. v.	174	39.1	0.088	0.018	7,879	165.3	8	
CCl_4	128	56.6	0.099	0.013	6,435	103.6	24	
ANIT	80	252.5	0.205	0.005	4,962	67.0	24	
Galactosamine	140	62.6	0.097	0.013	6,686	102.6	24	

C_0 : The plasma level at zero time. $T_{1/2}$: The half-life.
V_d : The aparent volume of distribution
K_{el} : The elimination rate constant from plasma
K_m : The metabolic rate constant. C_{max} : The maximum plasma level.
T_{max} : The time to reach the maximum plasma level. AUC : The area under the curve.
CCl_4 (0.5 mVkg, p.o.). Galactosamine (300 mg/kg, i.p.)
ANIT (150 mg/kg, p.o.) . Rats were pretreated with CCl_4, ANIT and galactosamine 24h prior to oral administration (Mean, n=4).

the number and substitution position of chlorine in the molecule. The inducing activity is varied compound to compound. For example, Prof. Yoshimura's group, Kyushu University showed that PCBs are divided into three types by the pattern of induction as shown is Table 11. The toxidity is also varied and some of MC type PCBs, especially 3,4,5,3′,4′-pentachlorobiphenyl, is very highly toxic and shows the increase of the activity of DT-diaphoase in cytosol, liver enlargement with fatty liver, and the atrophy of thymus and spleen.

PCDFs, polychlorinated dibenzofurans produced from PCBs, have been recently established as the real causal substances in Yusho disease. PCDDs, polychlorinated dibenzodioxins, especially TCDD, 2,3,7,8-tetrachlorodibenzodioxin, is an extremely highly toxic substance and the epidose of Seveso, Italy, is very famous. These compounds are also very active inducers. Recently, the studies on the mechanism of induction have been rapidly and deeply developed especially from genetical point of view.

Di-2(-ethylhexyl) phthalate (DEHP) one of the commonly used plasticizers, induces the content of cytochrome P-450 in microsomes in rats fed a 0.5% DEHP-diet. As shown in Fig. 6, the dose-response curves for liver weight and aminopyrine N-demethylase are parallel between PCBs or DEHP alone and the combination in various doses, but those for cytochrome P-450 are radiate. Therefore, it is estimated that the combined effect of DEHP and PCBs (a mixture of Kanechlor 400 and 500) on liver weight and aminopyrine N-demethylase are additive but the effect on cytochrome P-450 is synergistic. Aniline hydroxylase is mainyl induced only by PCBs.

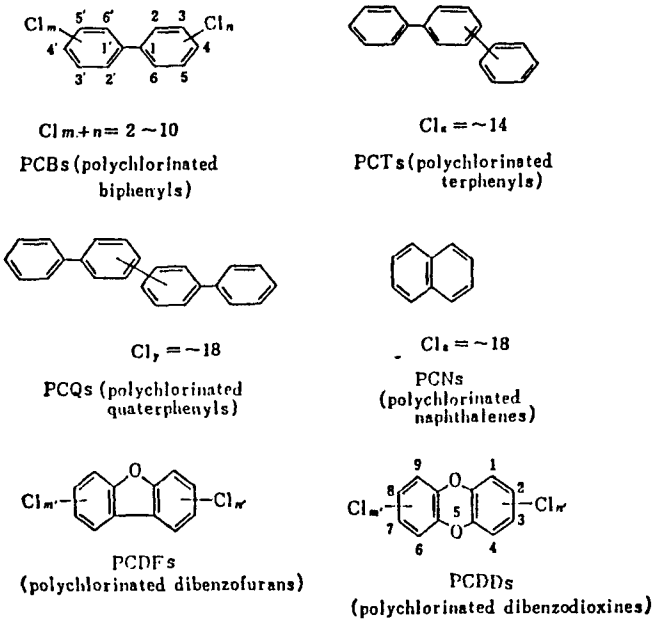
Liver enlargement is often observed in the course of general toxicity testing of various chemicals and it is considered to be one of the pathological changes useful as the index for the safety evaluation. However, it is reasonable to consider that this alteration may represent an adaptive repsonse of animals to the exposure to xenobiotics when it is accompanied by the stimulation of microsomal drug-metabolizing enzyme activity and when any other apparent

Table 11. Classification of PCBS by induction pattern

Number of chlorine	PB type	Intermediate type	MC type
2	4, 4' – 3, 3' –		
4	2, 4, 3', 4' – 2, 5, 2', 5' – 2, 4, 2', 4' –		3, 4, 3', 4' – (w)
5		2, 4, 5, 3', 4' – 2, 3, 4, 3', 4' –	3, 4, 5, 3', 4' – (s)
6	2, 4, 5, 2', 4', 5' – 2, 3, 4, 2', 3', 4' –		3, 4, 5, 3', 4', 5' – (s) 2, 3, 4, 5, 3', 4' – (w)
7	2, 3, 4, 5, 2', 4', 5' – 2, 3, 4, 5, 2', 3', 4' –		
8	2, 3, 4, 5, 2', 3', 4', 5' –		
10	2, 3, 4, 5, 6, 2', 3', 4', 5', 6' –		

(w) : weak, (s) : strong
(H. Yoshimura)

Table 11'.



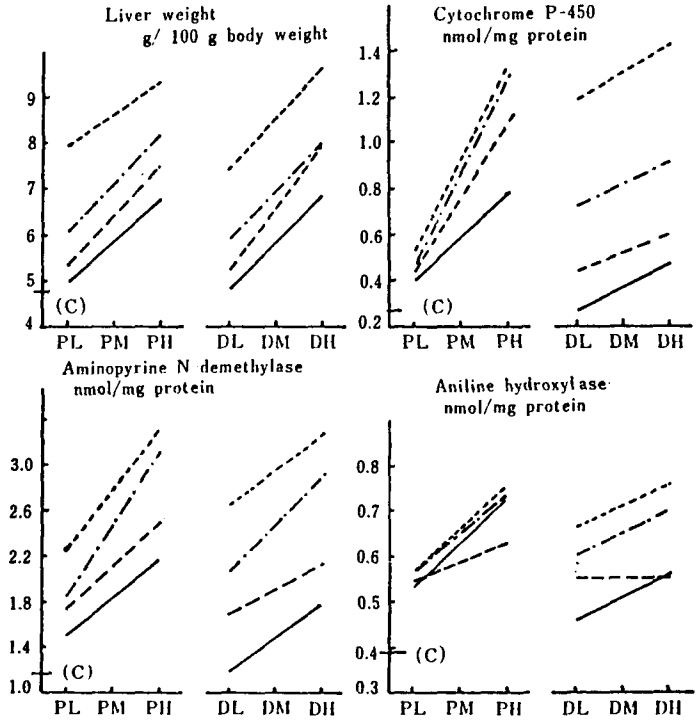


Fig. 6. Dose response curves for combination of PCB and DEHP
Dose-response curves for one chemical combined with other chemical of- - -high dose, — · — · mediam dose, or- — —low dose. —— Dose-response curve for PCB or DEHP alone.
(C) : Value of control (PO—DO group).

changes are absent in this organ. On the other hand, well-established hepatotoxins such as carbon tetrachloride also increase the liver weight moderately during the early phase of administration, although they reduce the activity of the microsomal enzymes in prolonged administration.

AF-2 (furylfuramide) which had been used as antimicrobial food additive in Japan till 1974 induced a marked liver enlargement in rats but reduced the activity of the microsomal drug metabolizing enzymes.

We examined the significance of liver enlargement induced by chemicals and compared the effects of PCBs, MC, AF-2, PB and DEHP. As shown in Fig. 7, the potency in inducing liver enlargement was on this order. The

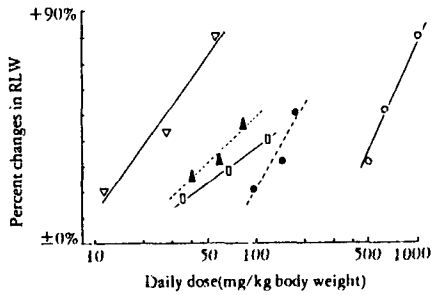


Fig. 7. Dose-response relationship of some chemicals on liver enlargement.
Male rats were treated with the chemicals for 5-7 days. The abscissa is on log scale indicating the daily dose and the ordinate represents percentage changes in relative liver weight vs. control values. The doses of the dietary administrated chemicals were calculated from the amounts of food intake. Each value represents the mean of three to six rats.
— ▽ — PCB (dietary), ... ● .... PB (i.p.)
— ▲ — MC (i.p.), ... ○ ..... DEHP,
— □ — AF-2 (dietary).

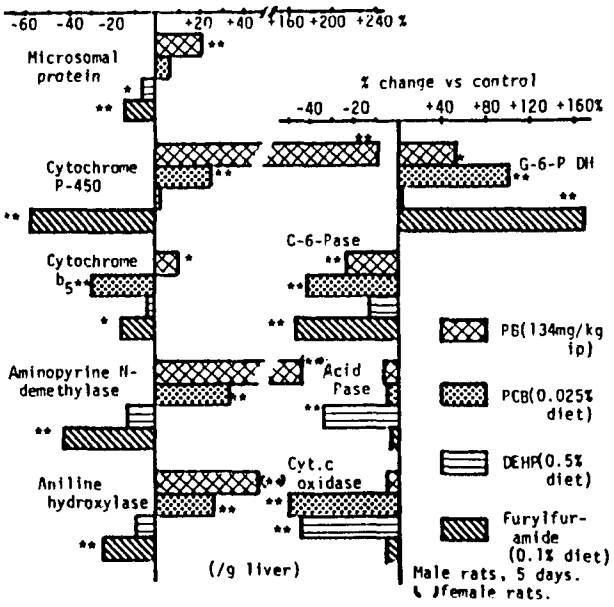


Fig. 8. Effects on hepatic enzymes (PB, PCB, DEHP, Furylfuramide)
Microsomal protein was increased by PB, but decreased by furylfuramide. Cytochrome P-450 was increased by PB and PCB, slightly increased by DEHP, but markedly decreased by furylfuramide. Glucose 6-phosphatase was decreased by every chemical. Glucose-6-phosphate dehydrogenase was increased by PB or PCB and markedly increased by furyfuramide. The effect of DEHP was generally weak.

biochemical parameters of the liver were affected in a different mamnner by these chemicals under the condition that caused the increase in the liver weight to the same level as shown in Fig. 8. The changes in the various hepatic parameters observed in this study showed approximately three patterns of response to five agents examined.

The patterns of drug metabolizing enzymes is not necessarily the detoxication but toxication. However, the patterns of response induced by PCBs, PB, or MC might be rather adaptive one, since no hepatic injury by themselves has been observed at the doses which caused the induction of drug metabolizing enzymes. While AF-2 -induced pattern of response might not be an adaptive one, since it is associated with the reduction of these enzymes, and moreover, the responses of the liver to AF-2 were markely different from those of PCB-, PB- and MC-type. Compared to these two patterns, DEHP-induced pattern showed third type which is similar to neither of the two. The effects of this compound were not so pronounced as those of other chemicals. The significance of this type of response is, at present, unknown. Thus, we have to consider the individual pattern of induction for each chemical.

Combined Effect of Inducer and Inhibitor

The toxicity of individual chemical has been extensively studied, but in evaluating the toxicity of environmental chemicals, the modification of toxicity of one chemical by co-existing other chemicals should be worth due consideration, because the environment has been contaminated by various kinds of chemicals at the same time. As mentioned above, PCBs is a widespread pollutant and a potent inducer of monooxygenases. In contrast, some foods such as fish are also contaminated by methylmercury, an inhibitor of monooxygenases. Cadmium is also popular contaminant and considered as causal metal for Itai-itai disease in Japan. This metal inhibits monooxygenase, too. It is interesting to learn what will happen, if the chemicals possessing the opposite action on the drug metabolizing mechanisms are mixed.

On the other hand, it is well documented as shown in Fig. 9 that bromobenzene which cause hepatic necrosis is converted by monoxxygenases to an active metabolite which covalently bound with macromolecules and further biotransformed to inactive metabolites,

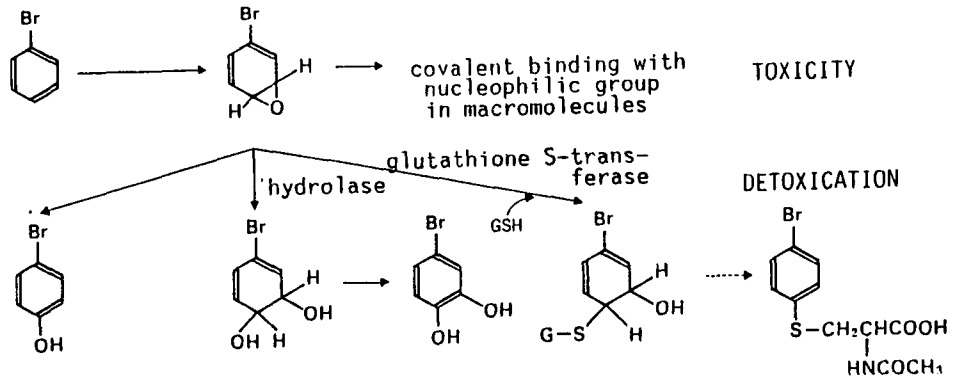


Fig. 9.

thus the toxicity of bromobenzene is modified by either inducer or inhibitor of monooxygenases.

The study of combined effect is a very complicated problem, because there is an infinite number of combinations in materials and doses. Although the chronic effect through oral route are of importance for considering the toxicity of environmental chemicals, we attempted, at first, to investigate the effect of methylmercury by parenteral administration on the inducing effect of PCBs in diet on the hepatic microsomal enzymes and the covalently binding of bromobenzene as a model compound.

The results show that the inducing effect of PCBs on hepatic microsomal monooxygenases was depressed by the acute parenteral administration of methylmercury. As shown in Fig. 10, the hepatotoxic action of bromobenzene was remarkably enhanced by PCBs, but the enhancing effect of PCBs was counteracted by methylmercury. The depletion of liver glutathione and the elevation of serum transaminases by bromobenzene were remarkably potentiated by PCBs. Methylmercury counteracted the effect of PCBs on serum transaminases but not that on liver glutathione. Table 12. shows that the amount of bromine covalently bound with microsomal protein after an injection of

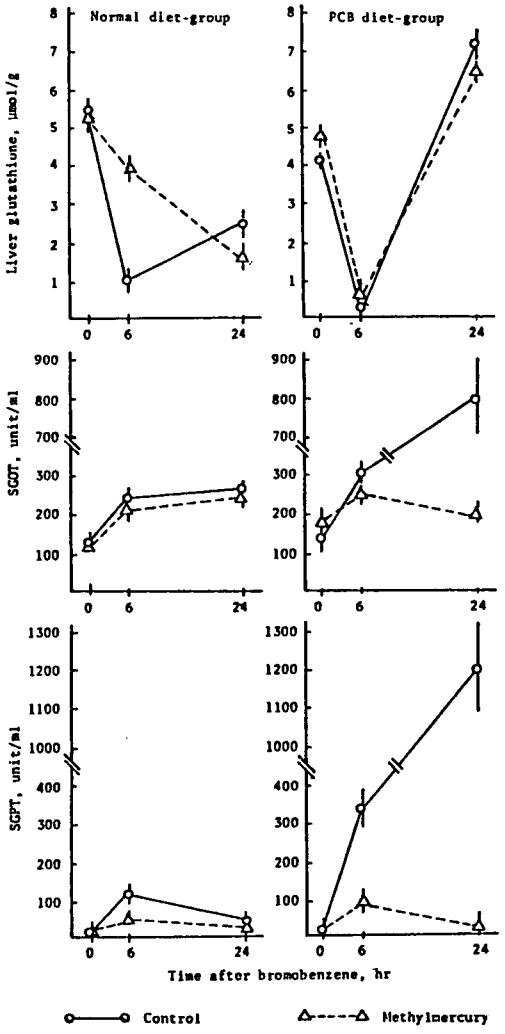


Fig. 10.

Table 12. Effect of pretreatment with PCB and/or methylmercury on the concentration of bromine bound with hepatic microsomes after an intraperitoneal injection of bromobenzene

Pretreatment	No. of rats	Br. ng/mg protein	As bromobenzene pmol
No bromobenzene	3	1.58 ± 0.70	
Control	5	4.00 ± 1.20	30.3
Methylmercury	5	3.47 ± 0.13	23.7
PCB	5	29.5 ± 8.16	349.2
PCB + methylmercury	5	11.4 ± 5.03	222.7

Rats were sacrificed 6 hr after an ip injection of bromobenzene, 5 mmol/kg.

bromobenzene. Table 13. shows that the radioactivity bound with microsomal protein after in vitro incubation of ^{14}C-bromobenzene with microsomes was fortified by PCBs pretreatment but depressed by the combining administration of methylmercury. Thus, it seemed that, if PCBs and methylmercury were administered in combination, they acted to reduce the toxicity of bormobenzene, even though each chemical has its individual toxicity.

The metabolism of chemical substances are not performed in only liver. The lung can also metabolize many drugs. Since the lung is the target organ of atmospheric pollutants involving carcinogenic chemicals in gaseous or aerosol forms, metabolism and dispositon of chemicals in lung has received much attention recently. Cd is known to be one of the potent inhibitors of the hepatic monooxygenases. We have studied the effect of Cd fumes on the lung microsomal drug-metabolizing enzymes of rabbits and have found that the activity of the enzymes were reduced after the short-term inhalation of Cd-oxide fumes. Cd fumes could modify the activity of xenobiotic enzymes in the lung and this may modify the metabolism and activity or toxicity of other atmospheric pollutants inhaled simultaneously. Fig. 11 shows the in vitro inhibitory effect of Cd on lung enzymes.

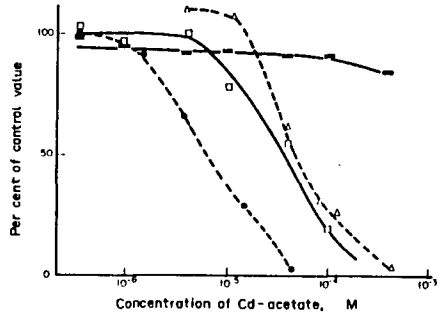


Fig. 11. Dose-related inhibitory action of Cd-acetate on monooxygenase activity of pulmonary microsomes of rabbits. Preincubation of microsomes was carried out for 20 min in the presence of Cd-acetate. The microsomal protein concentrations for the assays of cytochrome P-450 (□). benzo [a] pyrene hydroxylase (●), aminopyrine N-demethylase (Δ) and NADPH-cytochrome c reductase (■) were 0.43 mg/ml, 0.75mg/ml, 0.97mg/ml and 0.27mg/ml, respectively. Each point, expressed as percentage of control values represents the mean of three determinations.

METABOLIC ACTIVATION AND TOXICITY

The biochemical studies on the toxicities such as carcinogenicity or mutagenicity have been widely developed and showed that the various metabolic activation system played the important roles for inducing toxicity. Bromobenzene previously is also metabolized to epoxide as an active metabolite. I would like to show the metabolic activation of styrene somthing in

Table 13. In vitro metabolism and protein binding of bromobenzene incubated with hepatic microsomes of rats pretreated with PCB and/or methylmercury

Pretreatment	Phenolic metabolite		Binding with protein	
	10 min	20 min	10 min	20 min
Control	0.444	0.560	0.194	0.291
Methylmercury	0.222	0.328	0.032	0.076
PCB	1.550	1.779	0.548	0.868
PCB + methylmercury	1.197	1.377	0.247	0.368

Values are expressed as μmol/mg microsomal protein.

detail and review other cases. The work concerning with styrene was mainly performed by Prof. Watabe's group, Tokyo College of Pharmacy. The metabolism of styrene is shown in Fig. 12.

Styrene is an important source of plastics and a variety of informations have been accumulated concerning with the toxicity and metabolism from the view point of health control against industrial poisoning and exposure from plastic products such as tablewares.

Biotransformation of styrene into styrene glycol via styrene oxide (phenyloxirane), a mutagen and skin carcinogen, by rat liver

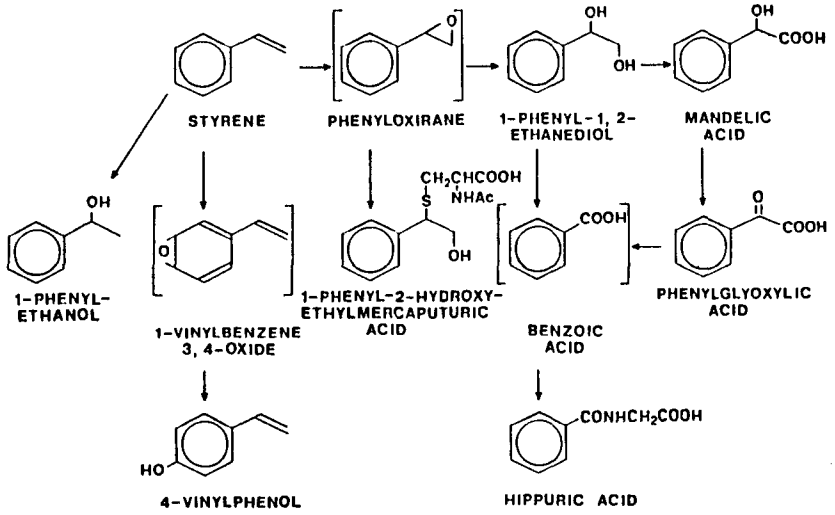


Fig. 12.

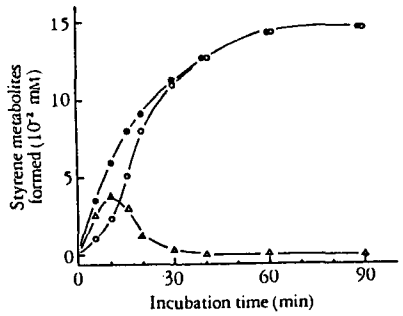


Fig. 13. A time course study of styene metabolism by rat liver microsomes in the presence of an NADPH-Generating system. Styrene (1 mM) was incubated aerobically with the microsomes (5.6 mg protein/ml) in air-tight flasks.
— Δ —, styrene oxide; — O —, styrene glycol
— ● —, styrene oxide + styrene glycol.

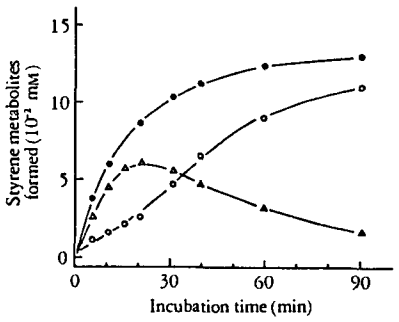


Fig. 14. A time course study of styrene metabolism by rat liver microsomes in the presence of an NADPH-Generating system and 3, 3, 3-Trichloropropene oxide as epoxide hydratase inhibitor. Styrene was incubated in the presence of the inhibitor (1 mM) under the same conditions as described in Fig. 26. Symbols are the same as used in Fig. 26.

microsomes in the presence of NADPH-generating system has been investigated. Both metabolites were identified by gas-chromatography-mass spectroscopy. As shown in Fig. 13, in the microsomal system, styrene oxide appeared only after a brief incubation and disappeared thereafter in spite of continued formation of styrene glycol. This phenomenon was rationally interpreted on the basis of evidence that during aerobic incubations, NADPH-dependent microsomal lipid peroxidation decreased P-450 activities selectively to a remarkable extent compared with epoxide hydrolase activities. As shown in Fig. 14, the addition of 3, 3,3-trichloropropene oxide (TCPO), a potent epoxide hydrolase inhibitor, to the incubation emdium prolonged the biological half-life of styrene oxide significantly, indicating one of major factors determining the biological level of the mutagen to be the relative ratio of these enzyme activities.

As shown in Table 14, styrene showed a weak mutagenic activity towards *Salmonella typhimurium* TA100 after being activated by the hepatic 9000 g supernatant fraction (S9) obtained from rats pretreated with MC or PB and fortified with an NADPH-generating system, in the presence of TCPO. The MC-pretreated rat liver S9 activated styrene more effectively than the PB-treated one as shown in Table 15. No mutagenic activity was observed when either TCPO or S9 was ommitted. Phenyloxirane induced mutations in TA100 and TA1535 cells in the absence of both S9 and TCPO. Analytical data of phenyloxirane and its hydrolytic product, 1-phenyl-1,2-ethanediol, formed in the mutation assay system for styrene indicated that the inducers used for hepatic drug-metabolizing enzyzmes enhanced both microsomal monooxygenase and epoxide hydrolase activities. As shown in Table 16, relative ratios of the enhanced monooxygenase to hydrolase activities were twice as high in MC as the control and 1.8 and 1.2 times in PB and PCBs, respectively, although the last enhanced both activities to the highest extent. Phenyloxirane was accumulated in the presence of TCPO in the styrene-activating system most signifi-

Table 14. Mutagenicity of styrene and phenyloxirane towards *Salmonella typhimurium* TA 100 in the presence and in the absence of S9 Mix.

Chemical (4mM)	Addition [a]		Survival (%)	Revertant colonies (per plate)	Induced mutation frequency
	S9 Mix	TCPO			
None	–	–	100	73	–
	+	–	100	80	–
	+	+	100	86	–
Styrene	–	–	5	66	–
	+	–	34	79	–
	+	+	30	105	91
Phenyloxirane	–	–	51	156	235
	+	–	87	90	17
	+	+	84	118	54

a) The S9 prepared from liver of rats pretreated with 3-methylcholanthrene was incubated with TCPO, when added, for 20 min 37° prior to the addition of chemicals, cofactors, and bacteria.
Other conditions for the assay were the same as described in Table 15.

Table 15. Mutagenicity of styrene towards *Salmonella typhimurium* TA 100 by its metabolic activation with S9 mix prepared from liver of rats pretreated with various inducers in the presence of TCPO

Inducer	Styrene (mM)	Survival (%) [a]	Revertant colonies (per plate)	Induced mutation frequency [b]
None	0	100	85	–
	2	100	85	–
	4	72	70	–
	6	14	70	–
PCB	0	100	92	–
	2	100	90	–
	4	76	78	–
	6	23	71	–
Phenobarbital	0	100	86	–
	2	94	82	–
	4	51	91	14
	6	9	91	83
3-Methylcholanthrene	0	100	81	–
	2	93	115	52
	4	30	100	90
	6	6	102	477

The liver S9 fractions prepared from rats pretreated with various inducers were preincubated with TCPO for 20 min at 37° prior to further 20 min incubation with the bacteria and styrene in the presence of an NADPH-generating system. The final concentration of TCPO was 0.3mM.

a) The value, 100%, represents about $1.7X10^8$ of surviving bacterial cells/ml incubation medium.

b) Values are ratios of numbers of induced mutanis to 10^8 of surviving bacterial cells. For instance, the induced mutation frequency 90 at 4 mM styrene in 3-methylcholanthrene-pretreated rat liver S9 mix was obtained from the following calculation : $(100-81)/0.4/0.3X1.74X10^8$.

Table 16. Biotransformation of styrene phenyloxirane and 1-phenyl-1, 2-ethanediol in the presence of TCPO by S9 mix prepared from liver of rats pretreated with various inducers

Inducer	Metabolites formed [a]				Relative EHase activity[b]	Relative ratio of monooxygenase to EHase activities
	PO (mM)	PE (mM)	PO + PE (mM)	(relative ratio)		
None	0.026	0.093	0.119	(1.0)	1.0	1.0
PCB	0.020	0.413	0.433	(3.6)	3.1	1.2
Phenibarbital	0.036	0.304	0.340	(2.9)	1.6	1.8
3-Methylcholanthrene	0.053	0.254	0.307	(2.6)	1.3	2.0

Styrene (4mM) was incubated with S9 Mix containing the epoxide hydratase inhibitor, TCPO, in the absence of bacteria under the same condition as described in Table 15.

a) PO : phenyloxirane and PE : 1-phenyl-1, 2-ethanediol.

b) The activity of epoxide hydratase (EHase) was shown as the relative ratio to that of untreated S9 (3.9 μmol of safrole oxide hydrolyzed/min/ml S9).

cantly when MC-pretreated rat liver S9 was used. However, the mutagenicity exerted by the metabolic activation of styrene could not necessarily be explained only by phenyloxirane since sums of both metabolites were smaller than the amount of the epoxide required for inducing the mutation in *Salmonella* and suggested a possibility of the presence of at least one more unknown mutagenic metabolite with an epoxide structure.

In order to elucidate this question, a putative intermediate in the metabolism of styrene to 4-vinylphenol, 1-vinylbenzene 3,4-oxide, was synthesized and examined for its obligatory intermediacy to the phenol, its physical properties and its mutagenicity toward *Salmolella typhimurium* TA98 and TA100. The 3,4-oxide had a half-life of 4.3 sec at pH 7.4 in an aqueous solution, and yielded 4-vinylphenol quantitatively without concomitant formation of any trace amount of 3-vinylphenol. This oxide had a potent mutagenicity toward the TA100 bacteria but not toward TA98 strain, whereas it showed

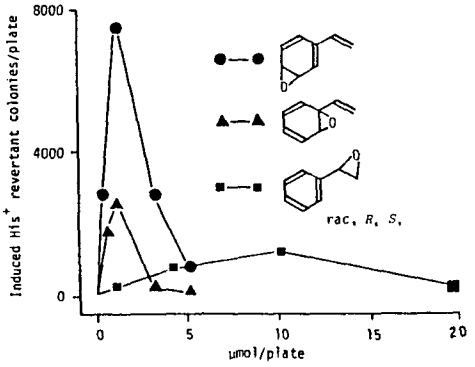


Fig. 15. Intrinsic mutagenicity of styrene oxides toward *Salmonella typhimurium* TA100. Each dose of a styrene oxide was divided into quarters and applied to the bacterial suspension every 5 min during the pre-incubation for 20 min. Abscissa shows total amounts of the oxide applied. Rac, R, and S represent racemic, R-and S-phenyloxiranes, respectively.

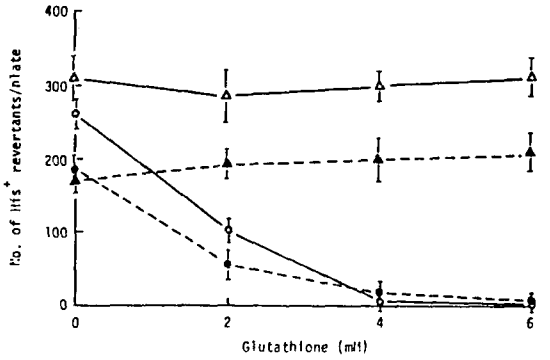


Fig. 16. Effects of glutathione on the mutagenicity of phenyloxlrane in the presence and in the absence of hepatic subcellular fractions from rats. Bacteria were pre-incubated with phenyloxlrane (4mM) and subcellular fractions (equivalent to 50mg each of the liver) in the presence of various concentrations of glutathione. Subcellular fractions : Δ , none : ▲, Ms : o, DSS ; •. S9. Spontaneous revertant colonies (103/plate) have been subtracted. Data are expressed as arithmetic means ± S. E. Decreased numbers of the induced revertants at 0mM glutathione in the presence of Ms and S9 compared with that in their absence were attributable to the metabolic inactivation of phenyloxirane by their microsomal expoxide hydratase during pre-incubation for 20 min.

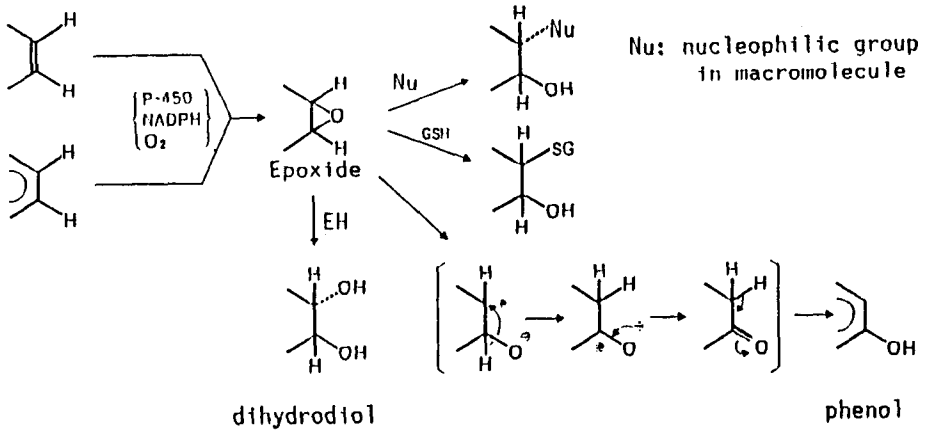


Fig. 17. Metabolic activation via epoxide.

a potent cytotoxicity to both of them. As shown in Fig. 15, His^+ revertant colonies induced by the 3,4-oxide were 7233/plate at a total dose of 1.0 umole/plate when it was applied in a sequential manner to the bacterial suspension during the pre-incubation of the testing system. Under the same conditions, benzo(a)pyrene 4,5-oxide and phenyloxirane showed 1283 and 1657 of His^+ revertant colonies/plate at 19 nmoles and 10 umoles/plate, respectively, as the maximal activities. The isomeric arene oxide, 1-vinylbenzene 1,2-oxide, had a longer half-life (1.63 min) than the 3,4-oxide at pH 7.4 in aqueous solution and was sepcifically rearranged to 2-vinylphenol. The 1,2-oxide also showed more potent mutagenicity to the TA100 strain bacteria than phenyloxirane but weaker than the 3,4-oxide. 4- and 2-vinylphenols were neither mutagenic nor cytotoxic to the bacteria at concentrations ranging up to 4 umoles/plate.

On the other hand, a comparative study on enzymic factors influencing the metabolic inactivation of phenyloxirane was carried out with respect to soluble glutathione S-transferase and microsomal epoxide hydrolase in S9 from a rat liver homogenate as shown in Fig. 16. The mutagenicity of phenyloxirane to TA100 was markedly reduced by S9 in the presence of glutathione but to a smaller extent in its absence. The retarding effect of glutathione on the inherent mutagenic activity of phenyloxirane was exerted by the soluble supernatant of S9 but not by microsomes. A GCMS study indicated that the effect of glutathione was attrigutable to the disappearance of the mutagen from the microbial assay system. The rate of the disappearance was 10-20 times as fast in the soluble supernatant fraction as in the microsomes when fortified with more than 4 mM glutathione. These results strongly suggest that in hepatic cells of the rat, cytosol glutathione S-transferase plays a much more important role than microsomal epoxide hydrolase in the detoxication of the metabolite, phenyloxirane.

The metabolic activation is one of the most atractive subjects in drug metabolism and toxicology and not a few research groups in Japan are progressing the remarkable studies. I would like to introduce some of them at last.

The metabolic activation pathways are mainly three groups. The first shown in Fig. 17 is the formation of epoxide shown in styrene, benzo(a)

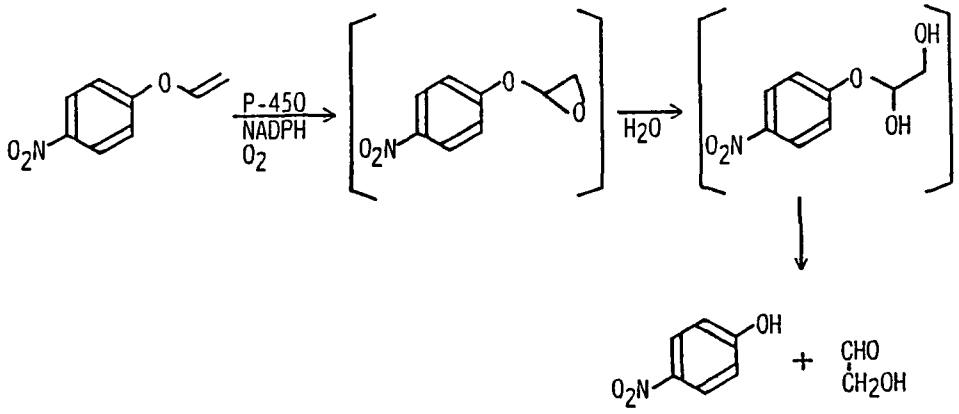


Fig. 18. Metabolism of p-nitrophenyl vinyl ether by rat hepatic microsomes.

pyrene, and bromobenzene. The epoxide, an electrophilic reagent, produced by cytochrome P-450 is able to covalently bind with nucleophilic functional group in macromolecules such as protein and nucleic acid in body. This is an initiation step of various toxicity such as carcinogenicity and mutagenicity. On the other hand, the epoxide will be conjugated with glutathione by non-enzymatically or glutathione S-transferase or hydrolyzed by epoxide hydrolase. These processes are detoxication mechanism.

By the way, it is important to utilize a suitable assay method for investigating any subject. For assay of olefinic epoxidation activity, no substrate is there for the spectrophotometric method and we studied p-nitrophenyl vinyl ether (NPVE) shown in Fig. 18. as a novel substrate for this purpose. NPVE was metabolized to p-nitrophenol and glycolaldehyde via an epoxide by rat hepatic microsomes. As shown in Table 17, the effects of monooxygenase and epoxide hydrolase inhibitors indicated that the oxdative metabolism of this compound was mediated by microsomal cytochrome P-450. Epoxide hydrolase plays a minor role, because a strong epoxide hydrolase inhibitor, TCPO, showed a weak inhibitory effect on the p-nitrophenol formation. The epoxy intermediate is so labile that the hydrolysis of the epoxide proceeds mostly nonenzymically even at a neutral pH. The induction study shown in Table 18

Table 17. Effects of monooxygenase and epoxide hydrolase inhibitors on the p-nitrophenol formation from p-nitrophenyl vinyl ether

Chemical	Concentration	p-Nitrophenol formed (nmol/mg protein/min)	(%)
None	–	1.62	100
SKF 525–A	0.10 mM	0.78	43
	0.01 mM	1.08	67
Metyrapone	0.10 mM	1.10	68
	0.01 mM	1.41	87
7, 8-Benzoflavone	0.10 mM	1.27	77
	0.01 mM	1.49	92
3, 3, 3-Trichloropropene oxide	1.0 mM	0.91 [a]	56
	0.2 mM	0.95 [a]	59
None	N_2/O_2 = 16/1	1.75	100
Corbon monoxide	CO/O_2 = 16/1	0.54	31

a) The enzymic reaction was carried out in a cuvette.

Table 18. Effects of monooxygenase inducers on the microsomal oxidation of vinyl ether derivatives and styrene

Pretreatment	Rate of microsomal oxidation (nmol/mg protein/min)						
	EVE	PVE	Me-PVE	NO_2-PVE	NVE	UVE	Styrene*
None	0.75 (1.0)	0.91 (1.0)	1.32 (1.0)	1.56 (1.0)	1.67 (1.0)	1.21 (1.0)	2.91 (1.0)
3-MC	0.63 (0.8)	1.37 (1.5)	1.55 (1.2)	1.89 (1.2)	2.12 (1.3)	4.22 (3.5)	3.34 (1.1)
PB	1.29 (1.7)	3.46 (3.8)	4.66 (3.5)	3.34 (2.1)	3.01 (1.8)	2.44 (2.0)	9.20 (3.2)
PCB (KC–400)	1.63 (2.1)	6.44 (7.1)	6.79 (5.1)	5.06 (3.2)	4.90 (2.9)	8.42 (7.0)	11.1 (3.8)

* Estimated from sttrene glycol formed.

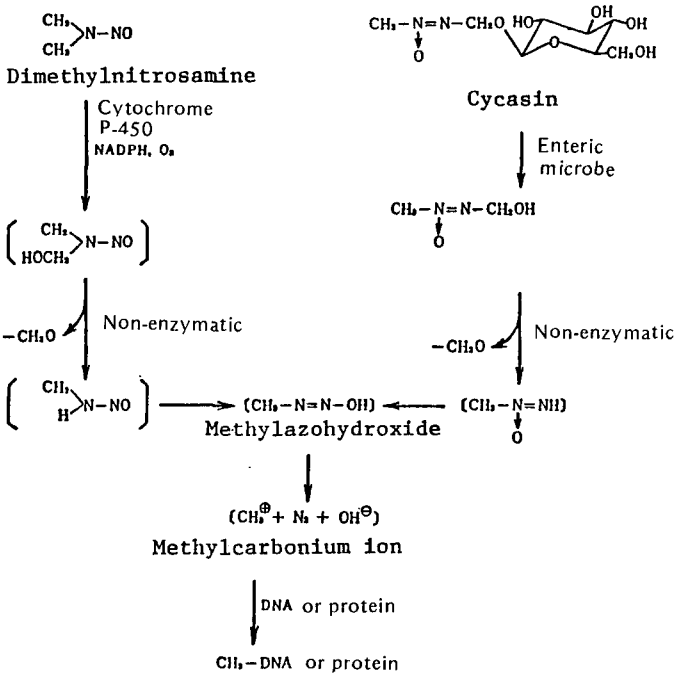
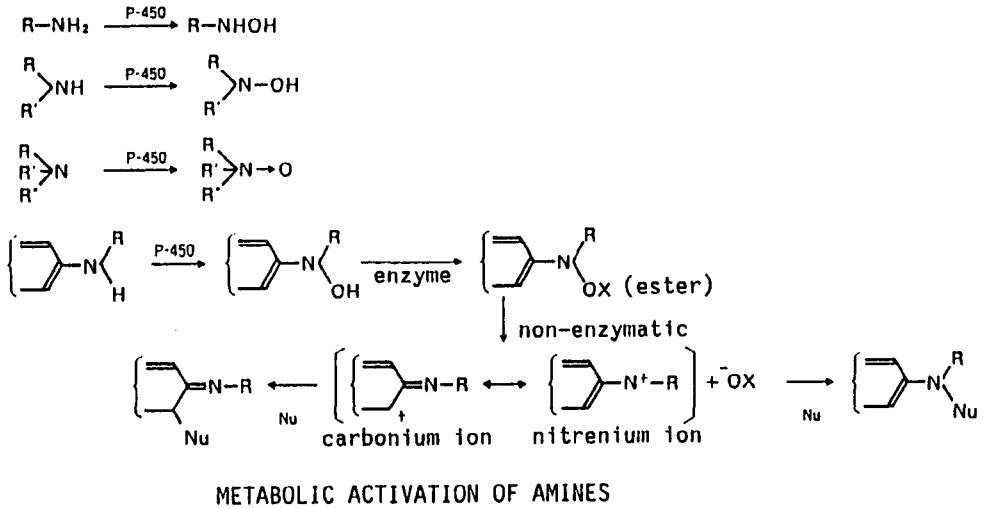


Fig. 19.



METABOLIC ACTIVATION OF AMINES

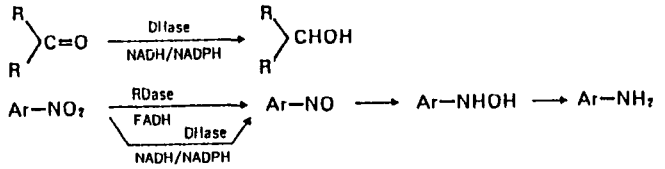


Fig. 20.

profiles for the micorsomal oxidation of NPVE were similar to those for the side chain oxidation of styrene in contrast to those for the O-demethylation of p-nitroanisole. Thus, a convenient and sensitive method for the assay of olefinic epoxidase activity was developed with NPVE as a substrate.

The second shown in Fig. 19 is the formation of alkyldiazohydroxide from dialkylnitrosoamine and cycacin, etc. Methylcarbonium ion produced from diazohydroxide reacts with DNA and results in carcinogenicity.

The third type of active metabolite shown in Fig. 20 is hydroxylamine ester produced from aromatic amines. Acetylaminofluorene is N-hydroxylated by hepatic cytochrome P-450 and converted to a hydroxamic acid and further to sulfate conjugate. Nitrenium ion produced from sulfate ester modify the nucleophilic group in DNA. In addition to sulfate ester, the ester with acetyl, serine, glucuronic acid are known as active forms. On the other hand, N-hydroxy-2-

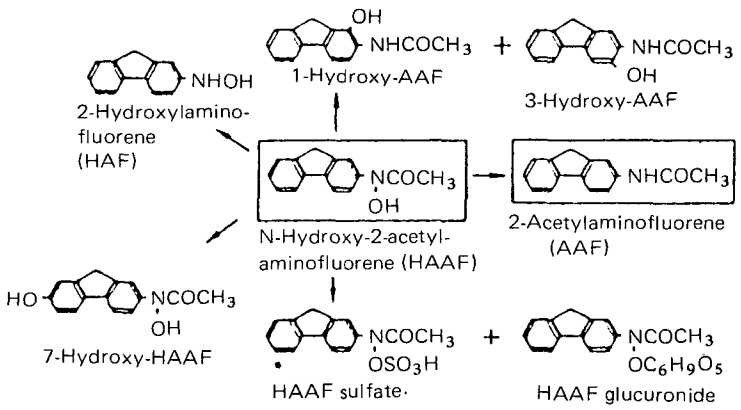


Fig. 21. (K. Tatsumi)

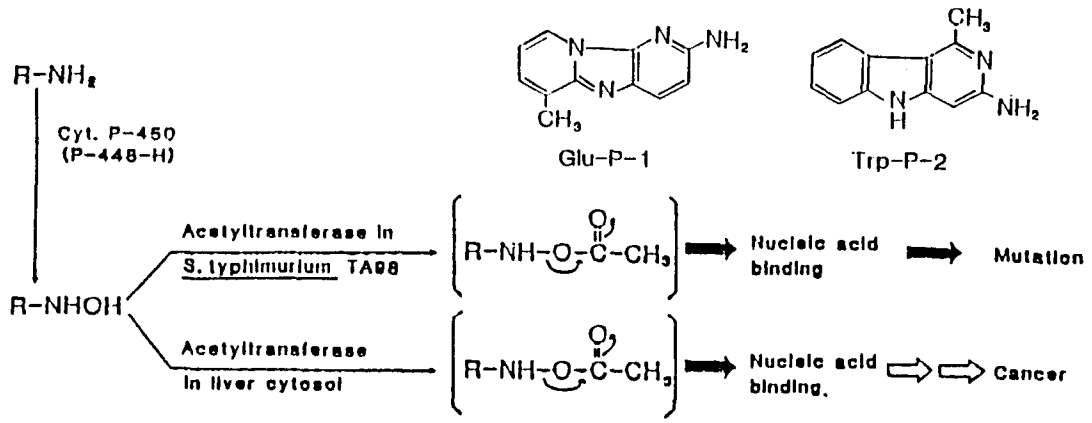


Fig. 22. Metabolic activation of Glu-P-1 and Trp-P-2 through o O-acetylation (R.Kato & T. Kamataki)

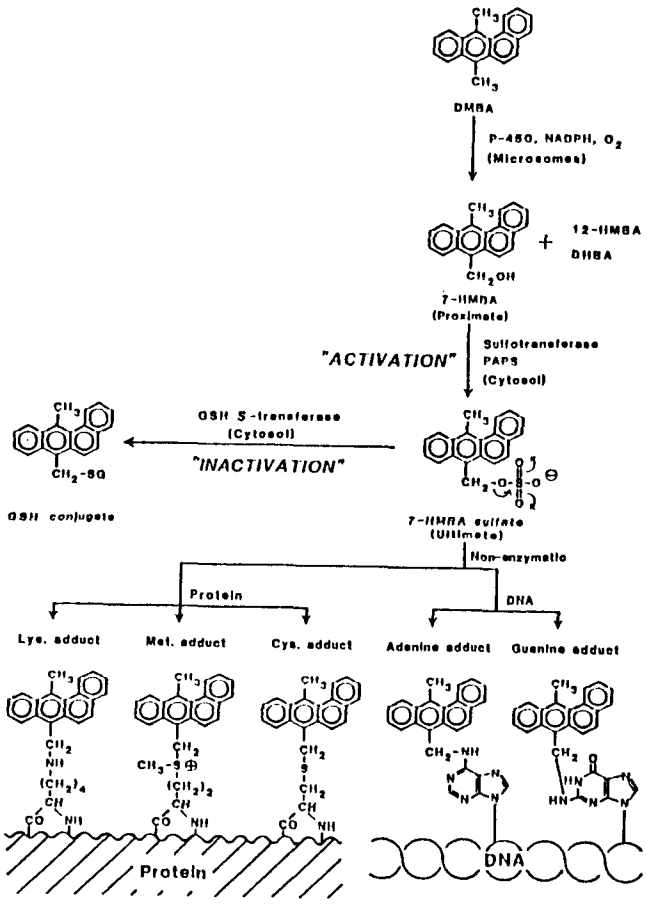


Fig. 23. Metabolic activation and inactivation of 7-HIBA by rat liver cytosolic sulfotransferase and GSH S-Transferase and mode of covalent binding of the active sulfate to biomacromolecules. (T. Watabe)

acetylaminofluorene is dehydroxylated with aldehyde oxidase and other enzymes as shown in Fig. 21. This reaction studied by Prof. Tatsumi's group, Hiroshima University, is an opposit reaction to activation. The amino acid pyrolysate such as Glu-P-1, 2 and Trp-P-1, 2 are very strong mutagens and also metabolically activated to N-hydroxylamine. These compounds are acetylated by CoA dependent O-acetyltransferase in liver cytosol and *Salmonella* as shown in Fig. 22 by Prof. Kato's group, Keto University.

In addition, the methylated polyaromatic hydrocarbons such as dimethylbenzanthracene is hydroxylated to hydroxymethyl derivatives and further esterificated with sulfate by the aid of sulfotransferase as shown in Fig. 23. This sulfate is an ultimate carcinogen and produce nonenzymatically the adducts with adenine and guanine residues in DNA and lysine, methionine or cysteine residue in protein. These modification is the initiation step of toxicity and studied by Prof. Watabe' group, Tokyo Pharmacy College.

EVALUATION OF SAFETY OF CHEMICALS

At last, I would like to consider the significance of metabolism study for evaluating the safety of chemicals. Table 19 shows the factors which should be considered in safety evaluation. In the phase of environments, chemicals are concentrated in biological bodies in the course of food chains, from algae, through small animals to big animals or from plants to animals. Anyway, the terminal is food stuff for human. Therefore, the study of biological concentration is important and this is just that of metabolism. After ingested into human body, chemicals act with body and express the activity and toxicity as mentioned previously. Toxicokinetic data are not directly show the toxicity but indispensable to understand the toxicity mechanism and evaluate the safety.

Table 19. Factors which should be considered in safety evaluation

	Step	Factor
Environment human	Release to environment	Amount, chemical and physical form
	Movement in environment	
	Physical trnasfer	Adsorption, diffusion, evaporation, etc.
	Chemical transformation	Photochrmical, microbiological, in plant change
	Biological concentration	Direct concentration
		Indirect concentration in food chain biodynamics, biological half life
	Exposure to human	via air, water, food pathways, amount, form, period
	Movement in human body	Absorption, distribution, excretion accumulation
		Metabolism (biotransformation) detoxication and activation (affected by environmental factors)
		Intetraction with constituents