Comparative Metabolism of Chlordimeform on Rat and Rice Stem Borer[*1]. Michihide MORIKAWA[*2], Shoichi YOKOYAMA[*3] and Jun-ichi FUKAMI (Laboratory of Insect Toxicology, The Institute of Physical and Chemical Research, Wakoshi, Saitama, Japan) Received July 4, 1975. *Botyu-Kagaku*, 40, 162, 1975.

31. クロルジメフオルムのラッテおよびニカメイガ幼虫における代謝

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本報ではクロルジメフオルム（*N*-(4-chloro-*o*-tolyl)-*N'*,*N'*-dimethylformamidine, CP）の作用機構解明の第1段階として ^{14}C標識 CP 塩酸塩を用いてラッテ，ニカメイガ幼虫の *in vivo*, *in vitro* における代謝実験をおこない，それぞれの代謝径路を推定し，その径路における両生物の質的量的相違点を明らかにし，CP の選択毒性の要因を代謝側面から解明しようと試みた．CP の代謝パターンは両種においてほぼ同様である．しかし吸収およびその代謝物の排泄はともにラッテの方が速かった．またラッテにおいてCPから（*N*-(4-chloro-*o*-tolyl)-*N'*-methylformamidine, DM）への分解と，*N*-formyl-4-chloro-*o*-toluidine から 4-chloro-*o*-toluidine を経て水溶性代謝物に至る分解径路への分解速度およびそれに関与する酵素活性がニカメイガ幼虫に比較して著しく高かった．一方ニカメイガ幼虫では，CP の表皮からの吸収が緩慢であり，かつその分解解毒の速度も非常に遅いために，生物活性があると考えられるCPおよび DM が徐々に蓄積されていくことが判明した．従って両種に対する CP の作用性の違いはおもに両種間の CP の分解量の差異が関与していると考えられる．またイネポット内に水中処理した CP はイネ体を通じてニカメイガ幼虫に浸透移行し，徐々に蓄積され，一部は代謝される．この代謝パターンは，ニカメイガ幼虫の表皮から吸収の場合と同じ結果が得られた．

Introduction

Chlordimeform (*N*-(4-chloro-*o*-tolyl)-*N'*,*N'*-dimethylformamidine) has been developed as an acaricide. The insecticidal nature of this compound to the rice stem borer as a noxious insect was investigated 24 or 48 hours after treatment by topical application, injection into the body of the borer and dipping the insect into its solution at a high concentration. However, as against the previous expectation, almost no insecticidal effect was observed[1,2,3].

On the other hand, when chlordimeform was directly sprayed on the rice plants in which the borer was kept freely, the borer evaded the eating of rice stem even in a lower concentration of this compound, thus there was almost no eating-away of the stem by the borer which thereafter died soon. At present, chlordimeform is used as a major insecticide against the borer[1,2,3]. Moreover, this compound is reported to exert low acute toxicity and no chronic toxicity against mammals[1,4].

Numerous reports have been published on the metabolism of chlordimeform in various species of animals and plants, such as goat, mouse[5,6] and young plants of apple[7] and grape-fruit[8], and *N*-(4-chloro-*o*-tolyl)-*N'*-methylformamidine, *N*-formyl-4-chloro-*o*-toluidine, 4-chloro-*o*-toluidine, *N*-formyl-5-chloroanthranilic acid, and 5-chloroanthranilic acid have been identified as the metabolites. Generally, the metabolic speed is prompt in mouse, while this compound is slowly absorbed and metabolized in the leaves and stems of plants. Whereas, as for the metabolism of this compound in the rice plant, Kuwano and Saito[9] observed *N*-formyl-4-chloro-*o*-toluidine, 4-chloro-*o*-toluidine, as the major metabolites. In the case

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of the *in vivo* experiments of insect and mites, the fate of chlordimeform was investigated in twospotted spider mites[10] and southern cattle ticks[11]. Metabolism of chlordimeform in these susceptible organisms proceeded at a slow rate when compared with houseflies[12].

Moreover, as for the metabolism of chlordimeform *in vitro*, the experiments were performed in the use of liver-microsomes and supernatant fraction of rat, and the metabolic mechanism was reported to be nearly the same as that for mammals and plants *in vivo*[13,14]. Homogenates of housefly abdomens and its microsomes converted chlordimeform to *N*-(4-chloro-*o*-tolyl)-*N'*-methylformamidine in the presence of oxygen and NADPH[12].

In the present report, as the first step for the clarification of the mechanism of action on chlordimeform, the metabolic experiment was performed *in vitro* and *in vivo* by the use of ^{14}C-chlordimeform HCl against rat and the borer, and each metabolic route was proposed, and clarification was made on the quantitative and qualitative differences of these creatures in each route.

At the same time, observation was made on the uptake of chlordimeform into the borer through the rice plant, and also, the metabolites thereafter were identified. Then, comparative review was made on the biological activities of the metabolites obtained in the above biological degradation against mammal and borer.

In summarizing the above results, discussion was made on the factors of the selective toxicity of chlordimeform.

Materials and Methods

Chemicals: ^{14}C-chlordimeform HCl (^{14}C-CP-HCl) labelled on tolyl-radical was supplied from Ciba-Geigy Ltd., Basel, Switzerland, and its radioactivity was 34.5 mCi/m mol. Upon starting each experiment *in vivo* and *in vitro*, ^{14}C-CP-HCl was dissolved in ethanol, and spotted on TLC of silica gel G as mentioned later, then developed by benzene-diethylamine (95 : 5) as mentioned later, and its purity was determined (the purity: 94.5%). Also, ^{14}C-metabolites used for the experiments for estimating metabolic route were biosynthesized by incubation for 3 hours with rat-liver microsomes + NADPH—system and ^{14}C-CP-HCl. At the end of the reaction, the incubation mixture was extracted with chloroform, and the extract was subjected to TLC of silica gel G which was developed with a mixture of benzene-diethylamine (95 : 5). After preparing the radioautography, radioactive metabolite zone was scraped off, and was put into 15AG, No. 4 glass-filter made by Ohuchi Rika Manuf. Co., Ltd., Tokyo, Japan, and then eluted with chloroform. Evaporation of the solvent gave the objective substance. As mentioned later, chemical structures of the isolated and purified metabolites were identified with co-chromatography, IR, and UV absorption spectra. The specific radioactivities of 8.60 mCi/m mol for ^{14}C-*N*-(4-chloro-*o*-tolyl)-*N'*-methylformamidine (^{14}C-DM), 8.6 mCi/m mol for ^{14}C-*N*-formyl-4-chloro-*o*-toluidine (^{14}C-NF), and 8.62 mCi/m mol for ^{14}C-4-chloro-*o*-toluidine (^{14}C-CT).

Unlabelled chlordimeform HCl (CP-HCl), Chlordimeform free base (CP) and 4-chloro-*o*-toluidine (CT) were obtained from Nihon-Nohyaku Co., Ltd., Tokyo, Japan, and they were purified by recrystallization (CP-HCl) and redistillation (CP and CT) before use. The purity was more than 98%. *N*-(4-chloro-*o*-tolyl)-*N'*-methylformamidine (DM) was prepared by heating CT and *N*-methylformamidine in the presence of $POCl_3$ at 50-60°C for 5 hours, in accordance with the amidine-synthetic method by Berdereck *et al.* (1959)[15]. The free base, which was obtained from the hydrochloride by extraction with benzene after neutralization (2N-NaOH), was recrystallized from *n*-hexane: M. P. 95-96°C, yield 36.5%, [calcd. ($C_9H_{11}N_2Cl$: 180.7), C: 59.16%, H: 6.07%, N: 15.32%, Cl: 19.47%. Found C: 59.05%, H: 5.97%, N: 15.32%, Cl: 19.47%]; IR (KBr) ν 3220 (νNH), 3050 (ν-CH), 1630 cm^{-1} (N=C-N); NMR ($CDCl_3$), τ 7.77s (3H), 7.05s (3H), 3.30d(H), 3.00d(2H), 2.56s (H). *N*-formyl-4-chloro-*o*-toluidine (NF) was obtained from CT with reflux in formic acid. It was recrystallized with ethanol; M.P. 122-3°C, yield 62%, [cacld. (C_8H_8NOCl: 169.6), C: 56.66%, H: 4.75%, N: 8.26%, Cl: 20.90%. Found C: 56.5%, H: 4.75%, N: 8.37%, Cl: 70.12%]; IR (KBr) ν 3210, 2880,

1640, 1510cm^{-1}; NMR ($CDCl_3$), τ 7.72s (3H), 2.20d (H), 1.60s (H), and each purity was 99%. Also, the free CP was obtained by neutralizing the hydrochloride solution with 2N-NaOH, and the mixture was extracted with benzene, and the extracts were evaporated to dryness under reduced pressure, and the residue distilled under the reduced pressure, gave the colorless free base (5 mmHg, 135-7°C). Its purity was more than 99%. 2-Diethylaminoethyl-2, 2-diphenylvalerate (SKF-525A) was obtained from Dr. R. Kato of National Institute of Hygienic Science, Tokyo, Japan.

Thin-layer chromatography (TLC): This was performed on the glass-plate (20×20 cm) of silica gel G (thickness: 0.30 mm) and silica gel HF 254 (thickness: 0.50 mm) made by Merck. The developer was benzene-diethylamine (95:5). CP, DM and CT were visualized on the plate by spraying the reagent of $PtCl_2$-KI, and NF was sprayed with the reagent of $K_2Cr_2O_7$-Conc. H_2SO_4 and coloring was made by heating at 110°C for 30 minutes. In such cases, CP showed light pink, DM showed light red, and CT showed light blue, also NF showed brown color. Moreover, each metabolite was detected under UV light (254nm). Usually, silica gel HF_{254} was used for the determination of chemical structures of CP and its related compounds.

IN VITRO EXPERIMENT

Enzyme preparations:

a) Preparation of enzyme solution:

The liver was taken out of 6 weeks old Sprangui-Dawly strain-male rat weighing 150-250g and its 20% homogenate (W/V) was prepared with the medium of 0.25M Sucrose-0.05M Na_2HPO_4-KH_2PO_4-ethylenediaminetetraacetic acid (EDTA) (pH 7.4) by a homogenizer of Potter-Elevhjen Type while being cooled. This 20% homogenate was centrifuged at 800×g for 5 minutes, and the precipitate (nucleus and debris) was removed, the supernatant solution was further centrifuged at 10,000×g for 10 minutes followed by 105,000×g for 90 minutes. This gave each mitochondria, microsomes, and each supernatant fractions. Mitochondrial and microsomal fractions were collected again, after suspending and washing with the above described medium. Each cellular fraction was adjusted on the volume with the medium for including the cellular fractional ingredient obtained from the fresh liver 200mg in 1 m*l* of enzyme solution, preparing each 20% mitochondria, 20% microsomes and 20% supernatant solution. Also, in a partial experiment, the supernatant solution after isolating the mitochondria was used as 20% microsomes+supernatant solution (soluble fraction).

In the case of the borer, the last instar of the male and female 200 larvae (about 64 mg/larva) the rice stem borer, *Chilo suppressalis* WALKER, used was raised in generation at Insectron of Rikagaku Kenkyusho (The Institute of Physical and Chemical Research, Saitama, Japan) were employed and 25%-homogenate (W/V) of whole larval body was prepared with the similar manner to that for preparing the homogenate of rat liver. Then, it was centrifuged fractionally to prepare 25% mitochondria, microsomes, supernatant solution, and microsomes+supernatant solution (1 m*l* of the enzyme solution contains the cellular fractional ingredient obtained from the equivalent solution to the larval tissue 250 mg). However, mitochondrial fraction was obtained with the centrifuge of 20,000×g for 10 minutes.

b) Enzymatic reaction:

The enzymatic reaction was usually performed with incubaton at 27°C for 60 minutes in the case of larva of the borer and the incubation for 60 minutes at 37°C in the case of the liver of rat under the air by putting NADPH (1.2×10^{-2}M) or NADH (1.4×10^{-3}M) as a cofactor, enzyme solution (1.0m*l*), 0.1M Na_2HPO_4-KH_2PO_4 buffer (pH 7.4) (1.0m*l*), and ^{14}C-CP-HCl (202μg), into 20m*l* of an Erlenmeyer flask. In the experiment for the inhibition of oxidative metabolism, SKF-525A was dissolved in 50% aqueous acetone, and 25μ*l* of the inhibitor was added to the incubation mixture.

After the end of the reaction, it was immediately cooled with ice, and kept at −20°C overnight for the analytical experiment. Upon analysis, the reaction mixture was warmed to the room-temperature, and transferred to a centrifugal tube (10m*l*) with the ground-stopper, then, the total volume of water-layer was made up to about 5m*l*

while washing the flask with the distilled water. Next, in washing the flask with 3.3 m*l* of chloroform, it was transferred to the centrifugal tube including the water-layer. After sufficient stirring and shaking, it was divided into chloroform-layer and water-layer by centrifuging the contents of the tube. This extraction with chloroform was repeated 3 times, and finally, exaction of 10m*l* of chloroform-layer and 5m*l* of water-layer were prepared, then, each 0.5 m*l* of these layers was taken into each vial, and 15 m*l* of scintillator was added to them. In the case of chloroform-layer, the chloroform was evaporated with N_2-gas stream and then, scintillator was added to it. The scintillator was prepared by dissolving 2,5-diphenyloxazole (DPO) (6g), 1,4-bis-2-(4-methyl-5-phenyloxazolyl) benzene (60mg) into a solution of toluene (1 liter), and methly cellosolve (500m*l*). The radioactivity was counted with a liquid-scintillation spectrophotometer (Model 3365, Packard Instrument Corp). The remaining chloroform-layer was dried over anhydrous Na_2SO_4, and was concentrated with evaporator (bath temperature: less than 40°C), thus by being divided into two, unaltered CP and its metabolites were separated with TLC. In this case, 20×20cm of a glass-plate of silica gel G (thickness: 0.30 mm) was divided into two, and the one side was measured on the metabolites by scraping the thin layer in accordance with the spot of radioautography, and the remaining side (20×10cm) was used for co-chromatography, in order to confirm the metabolites.

IN VIVO EXPERIMENT

A) Rat

a) Method of administration and handling:

Sprangui-Dawly strain-male and female rats (6 weeks old, average weight: 150g) were kept for fasting during the period of 15 hours, and were used for the experiment in a group consisting of 2 rats. ^{14}C-CP-HCl was dissolved into distilled water, and its 300mg (3.52 μCi) per rat was orally administered through the stomach-probe. This dosage corresponded to nearly LD_{50} of the oral dose of CP against rat[1,4,16]. After treatment, each rat was transferred into the metabolic cage of glass-funnel system (made by Takashima Shokai, Tokyo, Japan), thus, urine and feces were collected in time-course while giving the food and water freely. In this dose, the authors observed no death during the experiment, and only some spasm was observed immediately after the administration, but it was restored within a few minutes, thereafter, no abnormality was seen in the outer appearance. The urine-collecting bottle was cooled with ice at 0-5°C in order to prevent the degradation of the metabolites.

i) Analysis of urine:

Radioactivity was measured by adding the scintillator to aliquots of urine (each 0.2 m*l*, twice) collected in time-course. The remaining urine was extracted with chloroform of 3 times-volume as with the case of *in vitro* experiment, being divided into two layers of chloroform and water. Thus, the total radioactivity was measured by taking aliquots (each 0.5 m*l*, twice) of chloroform-layer and water-layer. Metabolites in chloroform-layer were isolated and analyzed in accordance with the method mentioned *in vitro* experiment.

ii) Analysis of feces:

^{14}C with the feces was dried under the reduced pressure to become a constant weight in the desiccator over P_2O_5, and then, it was pulverized in the mortar, its each 30 mg was taken for the measurement with humidity-burning system in accordance with Van-Slyke's method in application of Aronoff's method[17]. The formed $^{14}CO_2$ was absorbed in hyamine (1 m*l*) for one hour, and the radioactivity was counted by adding the scintillator (14 m*l*).

iii) Analysis of the tissue organs:

The liver, kidney, heart, brain, and blood plasma were collected from the rat after 96 hours of the administration of ^{14}C-CP-HCl. Each tissue was homogenated with a mixture of cooled acetone-water (4 : 1), followed by the isolation into the supernatant solution and the precipitate by centrifugation. From the supernatant solution, acetone was removed at 40°C. Aliquots (each 0.5 m*l*, twice) of the remaining water were added with 1 m*l* of Nissan Nonione-NS-210-solution (Nihon Yushi Co., Ltd., Tokyo, Japan) and the scintillator (14 m*l*), then radioactivity was measured. The radioactivity of the precipitate was measured in accordance with Van Slyke's method

after drying over P_2O_5 as in the case of the analysis of the feces.

B) Rice stem borer

a) Penetration experiment of ^{14}C-CP-HCl into the larval body of the borer through the integument:

In order to investigate the penetration transferrence of ^{14}C-CP-HCl into the larval body and the metabolic amount of whole insect larval body, 4th instar-larvae (30 mg/larva) of the borer reared in generations at Insectron of Rikagaku Kenkyusho (The Institute of Physical and Chemical Research, Saitama, Japan) were employed. Ethanolic solution (1 μl) containing 20μg ($8\times10^{-3}\mu$Ci) of ^{14}C-CP-HCl was applied on the part around the 3rd arthrodorsal face of the abdomen by a microsyringe, and after drying in the air, each 5 larvae were put into the test tube with ground-stopper, and it was kept at 25°C. This dose was 1/10 of LD_{50} at the elapse of 10 days after treatment. The treated 5 larvae in each section were used for the analysis after 22.5 minutes, 1.5 hours, 6 hours, 24 hours and 96 hours. Acetone (5m*l*) was used for the rinsing the larval surface and the whole test tube, then after drying the larval surface and the tube with N_2 gas-blowing, 2 m*l* of distilled water was added to tube and larvae. Again larvae and tube were dried with N_2 gas-blowing. Such rinsing with acetone and distilled water was repeated 3 times. The radioactivity recovered from the acetone and water-rinsing solution was regarded as the attached amount to the larval surface. In order to measure the amount of CP and degraded compounds being penetrated to the larval body, after the final drying with N_2-gas stream, glass-homogenizer was used, being homogenized in 5 m*l* of cold acetone-water mixture (4 : 1) per 5 larvae, and after centrifuging, acetone was removed under N_2-stream. Next, extraction was made with the same amount of chloroform, 3 times, and chloroform-extracted layer and water-layer were made up to 20 m*l*, respectively and quantitatively determined, then, isolation and analysis of ^{14}C-CP-HCl-metabolites were performed on chloroform-extracted layer and water-layer by the method mentioned in *in vitro* experiment.

b) Experiment of in vivo distribution of ^{14}C-CP-HCl penetrated through the integument of the larva of the borer:

In order to investigate the movement of ^{14}C-CP-HCl after its complete transference into the larval body of the borer, the time course-review was studied on ^{14}C-metabolites in 3 tissues, namely, the epiderm, digestive tube, and fat body of the treated larva of the borer. The analytic method was as follows. As with the above method, the site around the 3rd sectional part of the abdominal and dorsal phase of the larva of 4th instar weighing 30mg per larva was topically treated with 1 μl per larva of ^{14}C-CP-HCl-ethanol solution ($3.2\times10^{-2}\mu$Ci, 80$\mu g/\mu l$) diluted with unlabelled CP-HCl by using microsyringe. After being dried with N_2-gas stream, each 15 larvae were put into the test tube with the ground stopper which was allowed to stand at 25°C. After 24 hours, all the larvae were lightly rinsed with a small amount of acetone and distilled water each 3 times to remove substances on the surface of larval body. Each 15 larvae were put into a new test tube with the ground-stopper which was allowed to stand at room temperature. After a fixed time intervals (1, 2 and 4 days after treatment), on each one section (each 15 larvae), the inside of the test tube with the larvae were cleaned with acetone and distilled water 3 times, which lead to measure the amount of excreted matter. Thereafter, the larvae were dried in the air, and were dissected or anatomized under microscope, to divide into 3 parts, namely, the epiderm, digestive tube, and fat body. In this case, the extracted epiderm and the digestive tube were attached with a part of the fat body, therefore, washing was made lightly with acetone, and the washing added to the fraction of the above fat body was analysed. The epiderm, digestive tube, and fat body were each homogenated by adding the cooled acetone-water mixture (4 : 1). Then, with the same method as that of the above penetration experiment of ^{14}C-CP-HCl into the larval body of the borer through integument, acetone-removal and chloroform-extraction were performed, and all the radioactivities were measured, also and the metabolites were identified and quantitatively

analyzed.

c) *Uptake-experiment of ^{14}C-CP-HCl into the larval body of the borer through rice plant after treating in water*:

The larvae of the borer of the 4th instar were inoculated to seven leaf stage of the rice plant (IR-8-strain) planted in the Wagner Pot of 1/5000 are, and it was left in the green house up to the time when the larvae ate away the stem of the rice. In the pot, the depth of water was kept at 3cm, and ^{14}C-CP-HCl was treated in the water, being stirred well, for homogenization. The treated dose was ^{14}C-CP-HCl, 12mg (500μCi) per pot, its concentration in water becomes 20ppm, and the application was made at the ingredient 60g/are, and this dose is equivalent to 5 times volume of the usually treated dose at the 2nd generation of the larva on 3%-granular agent of chlordimeform. On 1, 2, 4, 8 days after treatment, each 10 rice stem eaten larvae were taken out, and the uptake and metabolites were analyzed in the similar manner to the penetration experiment from the larval surface into the body.

Identification of metabolites:

Chloroform-extracted materials obtained from the liver of rat by the method mentioned in *in vitro* experiment, were subjected to TLC (Silica gel HF_{254}), and the Rf value coinciding with that of an authentic sample was extracted with chloroform, the obtained each metabolite was examined with IR (Hitachi EPI-G_2 Type) and UV absorption spectra for the confirmation of the chemical structure of the metabolites.

RESULTS

A. *In vitro* metabolism of ^{14}C-CP-HCl by tissue homogenate of rat liver and rice stem borer

a) *Metabolism of ^{14}C-CP-HCl by rat liver tissue homogenate*:

^{14}C-CP-HCl was incubated with rat-liver homogenate, mitochondria, microsomes, and each fraction of supernatant fraction, and microsomes+supernatant (soluble fraction) (Table 1). Autoradiography of chloroform-soluble metabolites on silica gel G TLC showed 4 spots, namely, Rf 0.11 (A), 0.23 (B), 0.36 (C), 0.57 (D). "A" was identified as *N*-formyl-4-chloro-*o*-toluidine (NF), "B" was identified as *N*-formyl-(4-chloro-*o*-tolyl)-*N'*-methylformamidine (DM), and "D" was identified as unchanged CP by IR, UV absorption spectra and co-chromatography, as mentioned later. Also, "C" was identified as 4-chloro-*o*-toluidine (CT) by co-chromatography. In addition, 5 non-identified minor metabolites were obtained, they were, I (Rf: 0.02), II (Rf: 0.05), III (Rf: 0.14), IV (Rf: 0.44), and V (Rf: 0.69). Moreover, considerable amount of the radioactivity was found at the origin, and this has not been identified. The degradation of ^{14}C-CP-HCl in the presence of NADPH, added as a cofactor, was most remarkable with microsomes+supernatant fractions (soluble fraction), followed by with homogenate and with microsomes-fraction, and unchanged CP was 10.2%, 10.3% and 27.3%, respectively. However, mitochondrial and supernatant fractions showed no difference in the nonemzymatic reaction, and almost no enzymatic degradation was observed. The formation of DM, namely, *N*-demethylation of CP, was found to be dependent upon NADH or NADPH, and the rate was especially high in microsomes + supernatant fractions at the time of adding NADPH, reaching 42.8%, followed by homogenate, microsomes, mitochondria, each showing 24.0%, 20.5% and 17.2%. However, the supernatant fraction showed almost no DM, it was only 1.6%. The formation of NF was found in microsomes+NADPH-system (which were the highest contents), followed by microsomes+NADH-system, each showing 29.2% and 21.2%. The production of NF without enzyme solution (Control) showed 11.0%. Therefore, the formation of NF seems to be due to nonenzymatic hydrolysis of CP and also the enzymatic reaction. The formation of CT was increased by about 1.2-2.2 times on each fraction in the presence of NADPH, and the higher amount of formation was observed in the following order, namely, homogenate, microsomes + supernatant fraction, supernatant fraction, microsomes, mitochondria. However, the amount of the formation was only a few percentage. The amount of the substances at the origin thought to be the polar metabolites were, in the presence of NADPH, homogenate and microsomes, showing each 23.9% and 13.6%, other remaining substances showed only less than

Table 1. Metabolism of ^{14}C-CP-HCl by Cellular Fraction of Rat Liver homogenate in Presence and Absence of NADPH or NADH

Incubation Constituents			Radiocarbon Recovered as Indicated Products, %										
			Chloroform-soluble metabolites										Water-soluble products
Cellular fraction	*Cofactor NADPH[a]	NADH	Rf 0.00 Origin	0.02 I	0.05 II	0.11 NF(A)**	0.14 III	0.23 DM(B)	0.36 CT(C)	0.44 IV	0.57 CP(D)	0.69 V	
Control (Sucrose-buffer solution)	+	·	0.6	<0.1	<0.1	11.5	<0.1	<0.1	1.1	<0.1	86.2	0.3	0.2
	·	+	0.5	<0.1	<0.1	11.3	<0.1	<0.1	1.3	<0.1	86.5	0.2	0.2
	−	·	1.2	<0.1	<0.1	11.0	<0.1	0.2	1.3	<0.1	86.0	<0.1	0.1
Homogenate	+	·	23.9	1.2	<0.1	14.9	2.2	24.0	6.5	0.6	10.3	1.1	6.0
	−	·	3.9	<0.1	<0.1	4.8	<0.1	1.2	3.0	<0.1	86.5	0.2	0.4
Mitochondria	+	·	6.1	0.7	<0.1	17.5	<0.1	17.2	1.8	<0.1	83.2	2.3	1.2
	·	+	5.0	0.3	<0.1	13.5	<0.1	3.4	1.2	<0.1	75.8	0.4	0.5
	−	·	4.4	<0.1	<0.1	10.8	<0.1	0.4	1.5	<0.1	82.7	0.2	0.2
Microsomes	+	·	13.6	0.6	0.3	29.2	<0.1	20.5	3.1	<0.1	27.3	1.0	2.4
	·	+	9.9	0.8	<0.1	21.2	<0.1	10.9	2.5	<0.1	53.3	0.6	0.8
	−	·	8.4	<0.1	<0.1	17.3	<0.1	0.4	2.3	<0.1	71.2	<0.1	0.3
Supernatant fraction	+	·	0.4	<0.1	<0.1	11.5	<0.1	1.6	4.0	<0.1	81.8	0.3	0.4
	·	+	0.5	<0.1	<0.1	7.2	<0.1	0.7	3.5	<0.1	87.2	0.4	0.5
	−	·	0.5	<0.1	<0.1	10.4	<0.1	0.3	2.8	<0.1	85.4	0.3	0.3
Microsomes + Supernatant fraction	+	·	9.6	2.4	4.2	12.7	<0.1	42.8	6.2	<0.1	10.2	1.3	10.6
	·	+	5.2	<0.1	<0.1	16.8	<0.1	17.0	3.8	<0.1	55.0	0.8	0.4
	−	·	3.6	<0.1	<0.1	11.8	<0.1	0.9	3.5	<0.1	79.5	0.3	0.4

* Concentration of NADPH in 2 m*l* of reaction mixture: 1.2×10^{-3}M
Concentration of NADH in 2 m*l* of reaction mixture: 1.4×10^{-4}M

a +=present, − = absent ( )** represents the name of metabolites.

10%. The radioactivity of water-soluble products showed the maximum of 10.6% at the time when the supernatant fraction was added to microsomes +NADPH-system, being dependent on NADPH.

b) Effects of the concentration of NADPH, reaction time and inhibitor against the metabolism of ^{14}C-CP-HCl by rat liver microsomes +NADPH-system :

Fig. 1 shows the effects of the concentrations of cofactor NADPH on the formation of the major oxidative metabolites of ^{14}C-CP-HCl by liver-microsomes. Unchanged CP recovered from the reaction mixture showed 66.8% in 0.15×10^{-3}M of HADPH, 22.4% in 1.2×10^{-3}M of NADPH, and 2.6% in 2.4×10^{-3}M, thus the unchanged CP was lowered in accordance with the higher concentration of NADPH. The formation of DM was 2.1 % at 0.15×10^{-3}M of NADPH, 18.8% at 1.2×10^{-3}M, and 31.3% at 2.4×10^{-3}M, thus the formation was increased with the higher concentration of NADPH. Consequently, the formation of DM seems to be dependent on the oxidation alone.

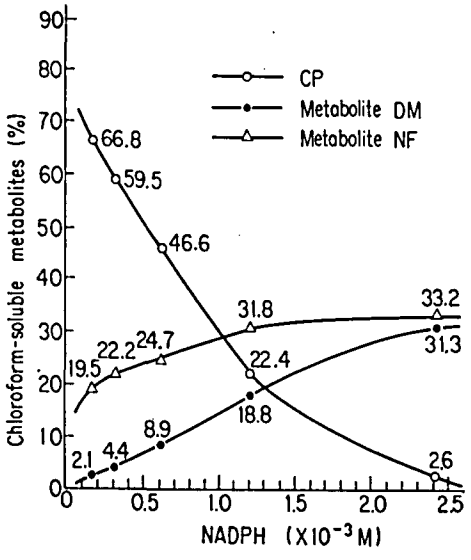

Fig. 1. Degradation of ^{14}C-CP-HCl by the concentration of NADPH at rat-liver microsomes. Open circle represents unchanged CP [*N*-(4-chloro-*o*-tolyl)-*N'*, *N'*-dimethylformamidine]; close circle, metabolite DM (*N*-(4-chloro-*o*-tolyl)-*N'*-methylformamidine], and also triangle circle metabolite NF (*N*-formyl-4-chloro-*o*-toluidine). Incubation time was 60 minutes.

NF was formed non-enzymatically, and partially by enzyme system. This enzyme system was dependent upon NADPH (Table 1, Fig. 1). However, the increasing rate of NF-formation due to the concentration of NADPH was slower than that of DM.

Fig. 2 illustrates the curves on the relationship between the reaction time and the amount of formation of NF and DM as the metabolites in the microsomes + NADPH-system. The amount

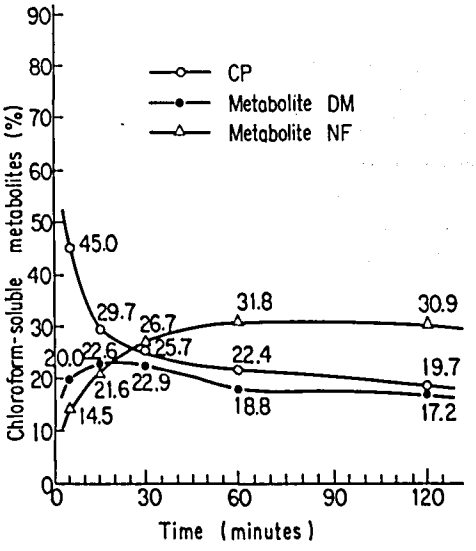

Fig. 2. The relationship between the reaction time and the amounts of metabolites on the degradation of ^{14}C-CP-HCl by rat-liver microsomes+NADPH-system. Circles were identical to those described in Fig. 1.

of CP recovered from the reaction mixture was 45.0% after 5 minutes, 22.4% after 60 minutes, and 19.7% after 120 minutes, revealing the decrease with the progress of time and the prompt reaction of oxidation at the initial period. DM formed by oxidation was 20.0% after 5 minutes, 22.9% after 30 minutes, and 18.8% after 60 minutes, revealing rather decreasing tendency. This means that further degradation of DM proceeds in the reaction mixture. On the other hand, NF was 14.5% after 5 minutes, 26.7% after 30 minutes, 31.8% after 60 minutes, revealing the increasing tendency, but no increase thereafter.

Table 2. Effect of SKF-525A on ^{14}C-CP-HCl Metabolism by Microsomes + NADPH-System Prepared from Rat Liver*

SKF-525A (M)	Radiocarbon Recovered as Indicated Product, %				
	Chloroform-soluble metabolites				Water-soluble products
	CP	DM	NF	CT	
10^{-5}	35.3	26.7	17.1	2.9	1.4
10^{-4}	59.5	8.9	13.1	2.3	0.7
10^{-3}	71.3	3.4	8.5	2.1	0.4
Non-addition	22.4	18.8	31.8	3.8	1.8

* The concentration of NADPH was same as that of Table 1.

As a result, DM was increased with the decrease of CP, and formed within a short time, thereafter, proceeded to the next step. While, NF showed the increase of the reaction under a partial enzymatic and non-enzymatic reactions, and the increase was continued even after no increase of DM-formation. Even the gradual decrease of CP stopped, the formation of NF was still increasing. These results was assumed to be shown that NF was formed not only from CP by oxidation but also from DM by hydrolysis.

Table 2 shows the effects of SKF-525A, an inhibitor of drug-oxidation on the metabolism by microsomes. The decrease in the degradation of CP was already observed even at the lower concentration of 10^{-5}M of the inhibitor. The decrease of the formation of DM was remarkable at the concentration of 10^{-4}M. Further increase of the concentration (10^{-3}M) of the inhibitor resulted a marked inhibition of CP-metabolism in general, and the degradation rate of CP (recovery rate from the reaction mixture: 71.3%) became nearly equal to that without enzyme (Table 1). In the case of the rat liver of which, the results summarized in Tables 1, 2, and Figs. 1 and 2, the biotransformation from CP to DM seemed to be involved with drug-oxidizing enzyme system from microsomes and also seemed to be affected with a kind of de-methylating enzyme.

c) *Metabolism of ^{14}C-CP-HCl, intermediate metabolites DM, NF, and CT in rat-liver microsomes and supernatant fractions*:

As already reported, CP became DM by demethylation. This is clear by the inhibition with SKF-525A which is an inhibitor of de-methylating enzyme, a kind of oxidizing enzyme of the drug. CP has a metabolic route of the direct enzymatic reaction into NF including non-enzymatic degradation. Attempts were made to investigate further changes of DM, NF and CT as intermediate metabolites in the rat liver, by the use of ^{14}C-labelled compounds. The degradation of ^{14}C-DM into various chloroform-soluble metabolites was observed in microsomes + NADPH-system, that is, the existence of the cofactor has enhanced the decomposition rate from 31.4% to 53.7%. Moreover, the further enhancement of degradation of DM (its rate: 83.0%) was observed by adding the supernatant fraction to this reaction system. This change is mainly the transfer reaction of choloroform-soluble metabolites into water soluble products (35.4%) (Table 3).

As a result of incubation of ^{14}C-NF, similar to the case of the above DM, the supernatant fraction showed a specially potent degradation into CT, and its formation-rate was equivalent to about 68.7% of the added radioactive carbon. On the other hand, the rate in microsomes + supernatant fraction was 53.7%, and 17.7% by microsomes alone. Consequently the formation of CT was proved to be independent upon NADPH. Also, at microsomes + supernatant fraction, the addition of NADPH caused the remarkable water-soluble products as with the case of the above DM (Table 4).

The degradation of ^{14}C-CT was dependent upon the microsomes + NADPH-system, and the existence of the cofactor induced more numerous water-soluble products, origin, I, II, and III. Especially, water-soluble products formed were

Table 3. Metabolism of ^{14}C-DM by Cellular Fraction of Rat Liver-homogenate in Presence and Absence of NADPH

Incubation Constituents		Radiocarbon Recovered as Indicated Product, %										
		Chloroform-soluble metabolites										Water-soluble products
Cellular fraction	NADPH	Origin*	I	II	NF	III	DM	CT	IV	V	Unknown**	
Microsomes	−	3.0	8.0	0.4	13.2	<0.1	68.6	2.9	<0.1	2.5	0.7	0.7
	+	7.0	12.7	4.6	15.7	<0.1	47.3	4.7	<0.1	0.9	0.6	6.5
Supernatant fraction	−	0.4	<0.1	0.3	7.7	<0.1	74.0	10.0	<0.1	<0.1	5.0	2.5
	+	0.6	0.3	10.6	7.8	<0.1	71.1	11.3	<0.1	<0.1	4.3	4.0
Microsomes + Supernatant fraction	−	2.4	0.3	0.3	8.7	0.9	64.6	8.9	<0.1	<0.1	10.5	3.4
	+	8.4	3.8	13.2	9.6	2.5	17.0	6.3	<0.1	<0.1	3.9	35.4

* The values of Rf in these metabolites and the concentration of NADPH were same as those of Table 1.
** At this experiment, unknown metabolites appeared at a part very near front of the Rf values.
+ = Present
− = absent

Table 4. Metabolism of ^{14}C-NF by Cellular Fraction of Rat Liver-homogenate in Presence and Absence of NADPH*

Incubation Constituents		Radiocarbon Recovered as Indicated Products, %									
		Chloroform-soluble metabolites									Water-soluble products
Cellular fraction	NADPH	Origin	I	II	NF	III	CT	IV	V	unknown	
Microsomes	−	0.7	<0.1	<0.1	79.6	0.6	17.7	<0.1	<0.1	1.1	0.3
	+	3.9	6.2	3.2	60.4	1.2	17.8	<0.1	<0.1	1.8	5.5
Supernatant fraction	−	0.2	0.2	0.2	25.8	1.7	68.7	<0.1	<0.1	2.1	1.2
	+	0.3	0.2	0.4	24.5	1.4	70.0	<0.1	<0.1	2.0	1.2
Microsome + Supernatant fraction	−	0.2	0.4	0.5	42.0	0.7	53.7	<0.1	<0.1	2.2	0.3
	+	1.5	2.1	2.8	29.5	1.4	43.2	<0.1	<0.1	4.4	15.1

* The detail was same as that of Table 3.

Table 5. Metabolism of ^{14}C-CT by Cellular Fraction of Rat Liver-homogenate in Presence and Absence of NADPH

Incubation constituents		Radiocarbon Recovered as Indicated Products, %								
		Chloroform-soluble Metabolites								Water-soluble products
Cellular fraction	NADPH	Origin	I	II	(II′)	(III′)	CT	IV	V	Unknown
Microsomes	−	1.2	0.1	0.2	5.5	2.5	72.2	14.0	<0.1	2.7	1.4
	+	5.3	5.7	2.0	6.0	4.9	43.1	5.7	<0.1	4.9	22.4
Supernatant fraction	−	0.7	0.2	0.2	0.9	3.5	85.8	3.6	<0.1	2.3	2.8
	+	1.1	0.3	0.2	0.9	1.7	85.8	3.8	<0.1	1.8	4.5
Microsome + Supernatant fraction	−	0.4	0.5	0.8	<0.1	3.1	84.9	<0.1	<0.1	6.6	3.8
	+	1.6	2.9	1.9	<0.1	1.9	33.7	<0.1	<0.1	5.5	52.5

( ): Substance obtained around the corresponding Rf-value, its Rf-value was 0.13 on II′ and 0.27 on III′
Other details were same as that of Table 3.

remarkable. The supernatant fraction was not affected to the metabolism of CT mostly, however, the co-existence with the system of microsomes +NADPH caused the highest transference into aqueous metabolites (52.5%). At present, the experiment is in progress on the nature or character of the products in water-soluble fraction, especially whether or not it is the conjugate (Table 5).

d) Metabolism of ^{14}C-CP-HCl in the homogenate whole larval bodies of the last instar of borer:

Table 6 shows the result of the incubation of ^{14}C-CP-HCl with microsomes, supernatant fraction and microsomes + supernatant fraction, obtained from the homogenate of the whole larval bodies of the last instar of rice stem borer, in the presence of NADH and NADPH as cofactors. A total of 6 spots were obtained, namely, the unknown substance V, origin, CT, NF, and DM as chloroform-soluble metabolites of CP. Most of CP remained unaltered except for non-enzymatic degradation of CP into NF. Especially, unlike the rat liver, almost no formation of DM was observed. Also, there was a little formation of the substances at the origin. The transfer by the system of microsomes+supernatant fraction in the presence of NADPH into water-soluble products was remarkably low as compared with that at the rat liver. Moreover, there was entirely no formation of the unknown substances, I, II, III, and IV. In the comparison on each cellular fraction, the degradation of CP showed no marked difference. The supernatant fraction was the most active among the cellular fractions, that is, the production of NF was 27.0% without the cofactor, the formation of DM and CT was a little higher than that of other fractions. Also, the addition of NADPH into the supernatant fraction reduced markedly the formation of NF as compared with non-addition. Therefore, there seemed to be not so marked NADPH-dependency in the metabolism of CP by the supernatant fraction. Microsomes showed lower metabolic rate than that of the supernatant fraction, and there was almost no difference between enzymatic and non-enzymatic reaction, namely, DM, was only 0.9-0.8%, NF was 15-18%, and CT was about 1%. However, microsomal reaction mixture in the presence of NADPH resulted a little higher metabolic rate than that in the absence of NADPH.

Table 6. Metabolism of ^{14}C-CP-HCl by Cellular Fraction of the Whole Larval Bodies Homogenate of Last Instar of Rice Stem Borer

Incubation Constituents			Radiocarbon Recovered as Indicated Products, %										
Cellular fraction	Cofactors* NADPH[a]	NADH	Chloroform-soluble metabolites** Origin	I	II	NF	III	DM	CT	IV	CP	V	Water-soluble products
Control (Sucrose-buffer solution)	+	·	0.3	<0.1	<0.1	18.2	<0.1	0.6	0.8	<0.1	79.1	0.9	<0.1
	·	+	0.9	<0.1	<0.1	12.8	<0.1	0.5	0.9	<0.1	84.4	0.4	<0.1
Microsomes	+	·	2.0	<0.1	<0.1	18.1	<0.1	0.9	1.2	<0.1	74.7	3.0	<0.1
	·	+	1.9	<0.1	<0.1	18.5	<0.1	0.8	1.0	<0.1	80.3	0.5	<0.1
	−	·	1.7	<0.1	<0.1	15.8	<0.1	0.8	0.8	<0.1	80.1	0.7	<0.1
Supernatnat fraction	+	·	0.7	<0.1	<0.1	20.6	<0.1	2.5	2.5	<0.1	72.9	0.3	0.5
	·	+	0.5	<0.1	<0.1	12.0	<0.1	2.2	1.4	<0.1	82.9	0.3	0.7
	−	·	0.8	<0.1	<0.1	27.0	<0.1	3.1	1.9	<0.1	66.3	0.3	0.6
Microsome + Supernatant fraction	+	·	0.7	<0.1	<0.1	15.2	<0.1	1.7	1.2	<0.1	80.7	0.2	0.3
	−	·	0.4	<0.1	<0.1	5.0	<0.1	1.9	0.7	<0.1	91.2	0.4	0.4

* The concentration of cofactors was same as that of Table 1, a+=present; −=absent

** The values of Rf was also same as that of Table 1.

Also, the metabolic rate in the microsomes+ supernatant fraction showed the lowest as compared with each fraction, especially, the degradation was low in the case of non-addition of NADPH, and NF was only 5%.

In summarizing the above results, each fraction showed very low formation of DM which is demethylated substance of CP, at the homogenate of the whole larval bodies of the rice stem borer.

B. *In vivo* metabolism of ^{14}C-CP-HCl by living rat and rice stem borer

a) Metabolism in rat:

i) Anaysis of urine

In oral administration of ^{14}C-CP-HCl into the living rat, CP and its metabolites excreted about 50% out of the administered amount after 24 hours in urine and the excreted rate was more than about 80% after 48 hours, 87.1% (male) and 95.4% (female) after 96 hours. The rate into chloroform-extracted layer was increased in time-course, reaching 15.2% (male). and 13.6% (female). The rate remaining in the water-layer was rather high, namely, about 40.6% after 24 hours, 71.9% (male) and 81.8% (female) after 96 hours (Table 7, Fig.3). Table 8 shows the distribution in time-course of chloroform-extracted layer against the doses. By means of TLC, major metabolites obtained were CP, DM, NF, CT, and

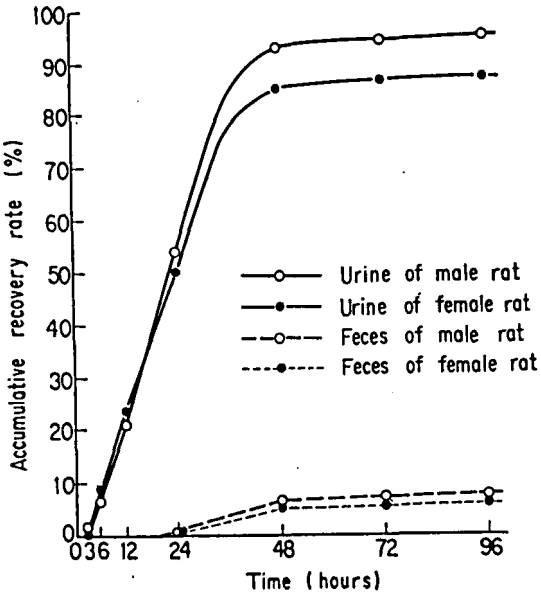

Fig. 3. Accumulative recovery rate of radioactive substances into urine and feces of 2 rat after oral administration of ^{14}C-CP-HCl.

Table 7. Excretion of ^{14}C-CP-HCl into Urine Following Oral Administration to Rat

Time (hours) after treatment	Sex	Accumulative recovery rate (%) of radioactive substance into urine against the dose	Relative rate (%) of accumulative recovery rate of water-layer and chloroform-exctracted layer	
			Chloroform	Water
3	♂	1.6	0.5	1.1
	♀	0.8	0.4	0.4
6	♂	6.9	1.8	5.1
	♀	9.0	2.9	6.1
12	♂	21.0	4.9	16.1
	♀	23.9	6.3	17.6
24	♂	54.5	13.2	41.3
	♀	50.8	10.2	40.6
48	♂	85.5	15.2	70.3
	♀	92.7	13.5	79.2
72	♂	86.8	15.3	71.5
	♀	94.9	13.6	81.3
96	♂	87.1	15.2	71.9
	♀	95.4	13.6	81.8

Table 8. Time course-distribution ($\times 10^{-2}$%) of Urinary metabolites against the Doses in the Metabolism *in vivo* in Rat on ^{14}C-CP-HCl in Chlorform-extracted layer

Time (hours) after treatment	Sex	Radiocarbon Recovered as Indicated Product ($\times 10^{-2}$%), %										
		Origin	I	II	NF	III	DM	CT	IV	CP	V	VI
3	♂	7.	1.	0.	3.	0.	33.	1.	0.	0.	0.	0.
	♀	14.	0.	0.	1.	0.	16.	2.	0.	0.	0.	0.
6	♂	14.	2.	0.	13.	0.	73.	18.	0.	1.	1.	1.
	♀	152.	8.	0.	19.	0.	47.	17.	0.	1.	1.	1.
12	♂	28.	3.	3.	23.	0.	135.	45.	3.	29.	19.	17.
	♀	60.	4.	3.	20.	0.	102.	57.	3.	28.	23.	15.
24	♂	119.	8.	5.	27.	10.	268.	199.	9.	92.	22.	20.
	♀	102.	9.	9.	17.	0.	105.	81.	5.	81.	12.	8.
48	♂	49.	7.	9.	17.	0.	22.	65.	4.	9.	5.	6.
	♀	85.	11.	15.	31.	0.	47.	84.	5.	20.	16.	6.
72	♂	1.	0.	1.	0.	0.	0.	1.	0.	0.	0.	0.
	♀	3.	1.	2.	1.	0.	0.	0.	0.	0.	0.	0.
96	♂	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.	0.
	♀	1.	0.	0.	1.	0.	0.	0.	0.	0.	0.	0.

* *In vivo* metabolites showed same Rf values and Co-chromatography as that of *in vivo* experiment as was shown in Table 1.

** Metabolite VI onyl showed high Rf value over 0.69 (the value of Rf on metabolite was 0.69)

the substances at the origin, as well as other 6 minor metabolites. The amount of these radioactive substances was in the following order, namely, DM > CT > CP > NF in male rat, and DM > CT > CP > NF in female rat. These major metabolites were gradually increased in urine after administration, reaching the peak after 24 hours, and decreasing thereafter. Among the metabolities excreted in the urine within 96 hours CP was equivalent to 1.3% (male) and 1.3% (female) of total administrated radioactive-carbon and also DM, 5.3% (male) and 3.2% (female) and NF, 0.8% (male) and 1.2% (female), and CT, 3.3% (male) and 2.4% (female), and metabolites at origin, 2.1% (male) and 4.1% (female), respectively. Most of ^{14}C in urine was water-soluble. This may be attributed that the metabolites in chloroform-extracted layer are transfered to water-layer after becoming highly water-soluble metabolites by undergoing degradation or conjugation metabolism in the tissues. From these results, absorption, metabolism and excretion of ^{14}C-CP-HCl in rat were proved to proceed promptly.

ii) Analysis of feces

The excretion of ^{14}C into the feces increased slowly up to 24-72 hours after the administration, but almost no increase was observed thereafter (Fig. 3). The recovery of ^{14}C was 5.76% in male and 5.24% in female after 96 hours, the recovery rates were far lower than that in urine.

iii) Analysis of tissue organs

Table 9 shows the distribution of ^{14}C-CP-HCl

Table 9. Distribution (ppm) of ^{14}C-CP-HCl and its metabolites in Various Tissue Organs Following Oral Administration to Rat

Liver	♂	0.1137
	♀	0.0778
Kidney	♂	0.0160
	♀	0.0210
Heart	♂	0.0006
	♀	0.0012
Brain	♂	0.0041
	♀	0.0051
Blood plasma	♂	0.1440
	♀	0.1071

and its metabolites into the liver, kindney, heart, brain, and blood plasma 96 hours after oral administration. In both male and female, there was very samll amount in the tissue, and the distribution of ^{14}C was within 0.0006-0.1137 ppm. The tissues detected to be high levels were the liver and blood plasma, and the lowest level was the heart.

b) Metabolism in the larva of rice stem borer:

i) Penetration into the body of ^{14}C-CP-HCl through the integument of the larva of the rice stem borer:

^{14}C-CP-HCl (20 μg) was topically applied, and time-course of penetrated amount through the integument was illustrated in Fig. 4. The rate of penetration proceeded slowly, and the radioactivity in the body reached more than about 50% after 24 hours (1440 minutes) and more than about 80% after 96 hours (5760 mintes). The radioactive substances in the body showed the gradual increase of the metabolites remaining in the water-layer after 24 hours and about 1/4 after 96 hours (Fig. 4). Next, by means of autoradiography, analysis was made on these chloroform-extracted layer in the body in the progress of time (Table 10). Besides CP, there were such metabolites as DM, NF and the metabolites at origin, and almost no detection was made on CT. These metabolites were increased in time-course, reaching 51.8% of the administered amount of ^{14}C-

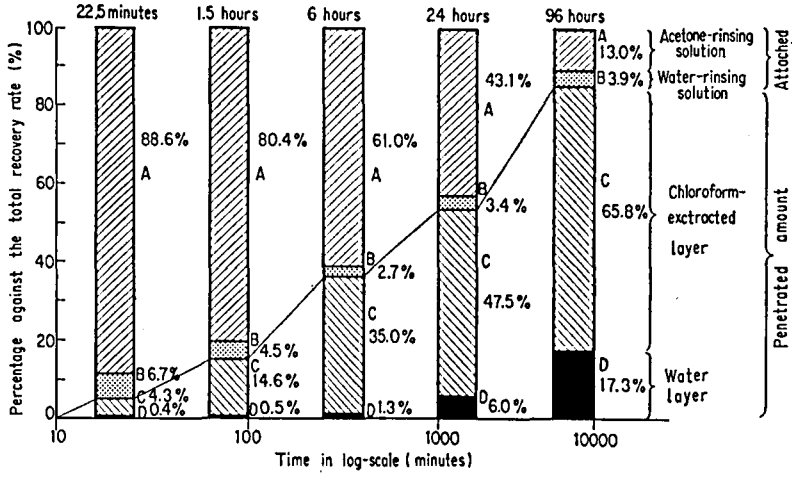

Fig. 4. The alternation in time course with penetrated amount of ^{14}C-CP-HCl in the body.
A represents acetone-rinsing solution in attached amount, and B, water-rinsing solution. C also represents chloroform-extracted layer in penetrated amount, and D, water layer.

Table 10. Time course-metabolism on Chloroform-extracted Layer in the Larval Body of Rice Stem Borer applied with ^{14}C-CP-HCl

Time (minutes) after treatment	Chloroform-soluble Metabolites (Radiocarbon Recovery Rate % against administered amount of ^{14}C-CP-HCl)				
	Origin	NF	DM	CP	unknowm stbstances
22.2	<0.1	<0.1	0.2	4.0	<0.1
90	0.2	0.3	0.5	13.0	0.2
360	2.0	2.3	5.8	23.8	1.1
1440	3.5	3.9	10.3	28.3	1.5
5760	5.1	10.2	14.9	31.7	3.9

CP-HCl after 96 hr (5760 minutes). Unchanged CP was about 92% in chloroform-extracted layer initially (22.2 minutes), but it gradually decreased to less than 50% after 96 hours, whereas, the rates of DM and NF in chloroform-extracted layer were increased, reaching each 23% (about 14.9% of the administered dose) and 15.5% (about 10.2% of the administered dose) from the initial 2-4%. The similar trend was observed on the metabolites at origin and the unknown substances.

ii) Distribution of ^{14}C-CP-HCl in the body penetrated through integument of the larva of the rice stem borer:

Table 11 shows the distribution of radioactive substances in the epiderm, digestive tube and fat body. In general, injection method is usually adapted to investigate the distribution of radioactive metabolites of ^{14}C-CP-HCl in the body rather than topical application. However the borer was found to be very difficult to adapt the injection method because size of the body was too small. Therefore, after 24 hours of

Table 11. Distribution (%) in the Body of Radioactive Substances penetrated into Larval Body of Rice Stem Borer*

			1 day after treatment	2 days after treatment	4 days after treatment
Excreted substances	Acetone-rinsing solution			9.4	7.7
	Water-rinsing solution			3.1	3.2
	Total			12.5	10.9
In the body	Epiderm	Chloroform-extracted layer	33.4	23.8	24.5
		Water layer	5.7	7.1	13.5
		Subtotal	39.1	30.9	38.0
	Digestive tube	Chloroform-extracted layer	11.0	7.4	7.6
		Water layer	2.7	3.4	5.0
		Subtotal	13.7	10.8	13.2
	Fat body	Chloroform-extracted layer	27.9	17.2	16.2
		Water layer	2.0	2.5	4.8
		Epidermal acetone-*** rinsing solution	8.1	5.1	4.8
		Digestive tube acetone-***rinsing solution	3.2	2.4	2.0
		Sobtotal	41.2	27.2	27.8
Unknown substances such as body-fluid**			6.0	18.6	10.1
Total			100.0	100.00	100.0

* % against the total in the body 24 hours after treatment (after cleansing the body-surface), that is, % against the added amount of the excreted matters after rinsing the body-surface to the total in the body

** The values of unmeasurable radioactive substances in the tissues such as body-fluid etc., were calculated by deducing the total of measured values at each organ from whole larval body of no dissection at each period.

*** As the fat body was not completely removed from the epiderm and the digestive tube, the removed epiderm and digestive tubes were lightly rinsed with acetone, being added to the fraction of fat body.

topical application of ^{14}C-CP-HCl, the surface of the larval body was rinsed with acetone and water, then the radioactive substances penetrated into the body was analyzed. The analysis of radioactive substances for the rinsing solution of acetone and water within 24 hours after the treatment of the topical application was not carried out at this experiment. Under the conditions of the present experiment, radioactive substances reached the digestive and fat body with 24 hours. Moreover, in chloroform-extracted layer of radioactive substances from the epiderm, digestive tube and fat body, the decrease was observed in 2 days after treatment at each tissues, while, in the period, 2 days after treatment and 4 days after treatment, nearly equal value was observed, namely, 23.8%-24.5% at the epiderm, 7.4%-7.6% at the digestive tube, and 17.2%-16.2% at the fat body. The excretion outside the body was similarly sustained after the 2nd day of treatment., Table 12, 13a, b, c and 14 show the results on CP and its metabolites in chloroform-extracted and acetone-rinsing layer of each tissue. The distribution-rate against the total in the body one day after treatment was the highest on CP in epiderm, and fat body, followed by the digestive tube. This tendency was similar even after 2 and 4 days. As for the metabolites at each tissues, DM was decreased in the progress of time at the epiderm, and NF was once increased 2 days after treatment, but decreased 4 days after treatment. On the other hand, CT and the metabolites at the origin were gradually and a little increased in time-course.

Topically applied ^{14}C-CP-HCl showed no-decrease after 2 days in view of fat body which is a further penetrated part. DM was reduced 4 days after treatment, NF was gradually decreased,

Table 12. ^{14}C-CP-HCl and its Metabolites in Chloroform-extracted Layer of Epiderm*

Time (days) after treatment	Chloroform-extracted Layer	Chloroform-soluble Metabolites**(Radiocarbon Recovery Rate % against Total Penetrated in the Body)									
		Origin	I	II	NF	III	DM	CT	TV	CP	V
1 day after treatment	33.4	1.7	0.5	0.5	1.9	0.9	7.0(21)	1.8	<0.1	19.1(57)	<0.1
2 days after treatment	23.8	3.5	0.5	0.7	3.0	0.5	4.4(18)	2.1	<0.1	9.0(38)	0.1
4 days after treatment	24.5	3.4	0.5	0.5	1.9	0.5	5.1(21)	3.8	<0.1	8.7(36)	0.1

* the detail was shown in Table 11.
** the values of Rf were also same as that of Table 1.
( ): When Chloroform-extracted layer was made to 100%, the percentages of CP and DM were shown.

Table 13a. ^{14}C-CP-HCl and its Metabolites in Chloroform-extracted Layer of Fat body.

Time (days) after treatment	Chloroform-extracted Layer	Chloroform-soluble Metabolites** (Radiocarbon Recovery Rate % against Total penetrated in the Body)									
		Origin	I	II	NF	III	DM	CT	TV	CP	V
1 day after treatment	27.9	0.8	0.2	0.4	2.6	0.4	3.8(14)	0.9	<0.1	18.8(67)	<0.1
2 days after treatment	17.2	1.2	0.3	0.3	1.3	0.3	3.4(20)	1.0	<0.1	9.4(55)	<0.1
4 days after treatment	16.2	1.4	0.2	0.2	1.5	0.2	0.8(5)	1.6	<0.1	10.3(64)	<0.1

* the detail was shown in Table 11.
** the values of Rf were also same as that of Table 1.
( ): the detail was shown in Table 12.

Table 13b. ^{14}C-CP-HCl and its Metabolites in Acetone-rinsing Solution of Fat body.

Time (days) after treatment	Acetone-rinsing solution	Acetone-soluble Metabolites** (Radiocarbon Recovery Rate % against Total penetrated in the Body)									
		Origin	I	II	NF	III	DM	CT	IV	CP	V
1 day after treatment	8.1	0.2	<0.1	0.2	0.8	0.2	1.1(14)	0.3	<0.1	5.5(68)	<0.1
2 days after treatment	5.1	0.4	<0.1	0.2	0.4	<0.1	1.0(20)	0.3	<0.1	2.8(55)	<0.1
4 days after treatment	4.8	0.5	<0.1	<0.1	0.4	<0.1	0.3(6)	0.6	<0.1	3.0(63)	<0.1

* The detail was shown in Table 11.
** The values of Rf were also same as that of Table 1.
( ): The detail was was shown in Table 12.

Table 13c. ^{14}C-CP-HCl and its Metabolites in Acetone-rinsing Solution of Digestive tube*

Time (days) after treatment	Acetone-rinsing Solution	Acetone-soluble Metabolites** (Radiocarbon Recovery Rate % against Total Penetrated in the Body)									
		Origin	I	II	NF	III	DM	CT	IV	CP	V
1 day after treatment	3.4	0.3	<0.1	<0.1	0.7	<0.1	0.6(18)	0.3	<0.1	1.1(32)	0.4
2 days after treatment	2.4	0.2	<0.1	<0.1	0.2	<0.1	0.3(13)	0.2	<0.1	0.9(38)	0.6
4 dyas after treatment	2.0	0.2	<0.1	<0.1	0.2	<0.1	0.2(15)	0.2	<0.1	0.8(40)	0.3

* The detail was shown in Table 11.
** The values of Rf were also same as that of Table 1.
( ): The detail was shown in Table 12.

Table 14. ^{14}C-CP-HCl and its Metabolites in Chloroform-extracted Layer of Digestive tube.*

Time (days) afetr treatment	Chloroform-extracted Layer	Chloroform-soluble Metabolite** (Radiocarbon Recovery Rate % against Total penetrated in the Body)									
		Origin	I	II	NF	III	DM	CT	TV	CP	V
1 day after treatment	11.0	0.5	0.2	0.2	2.2	0.3	1.5(14)	0.9	<0.1	3.6(39.6)	1.4
2 days after treatment	7.4	0.6	<0.1	<0.1	0.76	<0.1	0.8(24)	0.6	<0.1	2.7(36)	1.5
4 days after treatment	7.6	0.9	0.17	0.17	0.9	0.17	0.8(11)	0.9	<0.1	2.8(37)	0.8

* The detail was shown in Table 11.
** The values of Rf were also same as that of Table 1.
( ): The detail was shown in Table 12.

showing nearly a constant level 2 days after treatment and thereafter. CT and the metabolites at the origin were increased in time-course slightly as in the case of epiderm.

As a result, the distribution of metabolites in the epiderm, and fat body showed the highest level in the following order, namely, DM>NF>CT, at the early period after treatment, and with elapse of time, the following order of metabolites is DM>CT>NF, thus revealing some progress of metabolism in time-course.

Whereas, as for the radiocarbon substances

reaching digestive tube, ^{14}C-CP-HCl was metabolite via epiderm, and fat body. Therefore, the amount of CP was much lower in digestive tube in comparison with that of epiderm, and fat body. The pattern of the metabolism showed the following order quantitatively one day after treatment, namely, NF>DM>CT and CT>NF>DM in 4 days treatment. From these results, it has been clarified that the radioactive metabolites which was further degraded by other tissues organs such as epiderm, and fat existed in digestive tube.

As for ^{14}C of organic solvent extracted-layer in the body, remarkably high levels were observed on CP and DM showing the insecticidal activity[1,4,16]. In the table 11 and 12 showing distribution in the body of radioactive substances penetrated into larval body of the rice stem borer, percentage of chloroform-extracted layer of epiderm was 33.4% and those of CP and DM are 19.1% and 7.0% respectively. When chloroform-extracted layer is made to 100%, CP and DM become 57.0% and 21%. The sum of the latter is calculated to be 78.0%. The sums of CP and DM in the table 12, 13a, b, c, and 14 were subjected to the above calculation. The sum of CP and DM was 81% (chloroform-extracted layer), and 82% (acetone-rinsing solution) in fat body and also 53.6% in digestive tube among all the tissues one day after treatment, and 57% in epiderm, 69% (chloroform-extracted layer) and 69% (acetone-rinsing solution) in fat body and 48% in digestive tube even after 4 days, therefore, it was very slowly metabolized and its speed was delayed in the progress of time. From the result, CP was proved to be gradually penetrated in the body after topical application to the larvae of the rice stem borer, and degraded in the body, becoming such major metabolites as DM, NF, CT, and metabolites at the origin. However, there was very few excretion after penetration in the body, being proved to remain in the body. Also, the speed of the metabolism and degradation was rather slow. As is shown in the Fig. 4, Tables 11, 12, 13a, b, c and 14, which show the quantitative distribution of metabolites, considerable amount of CP and DM had been accumulated in the body.

iii) Uptake of ^{14}C-CP-HCl into the larva of rice stem borer through the rice plant after treating in water

Table 15 shows the result of the measurement of the uptake of ^{14}C-CP-HCl into the larvae of the 4th instar of the rice stem borer through the rice plant after treating in water. In the case of the species IR-8 as the tested rice plant, the total amount of CP and its metabolites taken from the larval body of the rice stem borer in the rice plant reached the maximum at nearly 24 hours after treatment, keeping a same tendency up to 4 days after treatment, but after 8 days, a decreasing trend was observed. While in the case of the species of "Kameji" of the rice plant, the maximum amount was observed in 4-8 days after treatment, therefore, some difference on showing the maximum value was observed by the different species of rice plants although a tendency was similar. The rates of metabolites remaining in

Table 15. Time Course-alteration of Uptake of Radioactive Substances into the Larval Body of Rice Stem Borer through Rice Plant*

	Days after treating the soil			
	1	2	4	8
The total amounts of CP-HCl and its metabolites on the Uptake into larval body (μg)	4.07×10^{-2} (91.7 : 8.3)**	4.43×10^{-2} (84.8 : 15.2)	4.55×10^{-2} (79.3 : 20.7)	3.18×10^{-2} (73.2 : 26.8)
Concentration (ppm) in the body per larva	1.36	1.48	1.52	1.06

* The subject rice Plant was IR-8 species. In the species of kamedi, the uptake 8 days after treatment was increased.

** The ratio of chloroform and water-extracted layer on ^{14}C-CP-HCl and its metabolite (%)

water-extracted layer were increased in the progress of time, reaching 26.8% 8 days after treatment, although the rate was only 8.3% 1 day after treatment, thus showing the same trend as that in the case of topical application as is shown in Fig.4 and Table 10. Table 16 shows the metabolic pattern of chloroform-extracted layer by TLC. As shown in the Table, besides CP, metabolites such as NF, DM and also "spot I" were observed, but CT was not observed. In view of the rate of each product in time-course, there was almost no change in 1-4 days after treatment, and either one showed the highest rate of CP, over about 1/2 of all, followed by DM, and the metabolites of the origin showed 10-20%. Such a trend of no change on the rate of each metabolite for 4 days after treatment was different from that in the case of the topical application. This seems to be due to the fact that in the present experiment CP and its degraded metabolites always entered newly into the larval body entered through rice stem. After 8 days of treatment, CP was reduced to about 22.8%, and degraded DM showed increasing to 20.3%. These obtained results was assumed to be showed that the amount of CP newly penetrated into larval body through rice stem decreased, thereby, the metabolism in the body progressed markedly.

As for the another reason, generally, CP showing insecticidal activities which enter into larval body through rice stem is considered to be due to the direct contact an eating with the rice stem, and the larvae affected with CP become inactive, and there after evaded the eating of rice stem (in fact, the larval body-weight per one body was reduced 8 days after treatment rather than that in 1 day after treatment)[1,4]. From these phenomena, oral CP-amount seems to be reduced.

Identification of metabolites:

In order to confrim the metabolites, an attempt was made to isolate the metabolites by the use of the system of rat-liver microsome+NADPH-system showing the highest metabolic activity. Into each one of 20 Erlenmeyer flasks (50 m*l*) with ground-stopper, each CP-HCl (2 mg: 100 μl ethanolic solution), NADPH-NADH (about 1.3×10^{-3}M), and 20 m*l* of 20% microsomes solution were added, and incubated at 37°C for 3 hours. Soon after, they were cooled with ice. Each 20m*l* of the reaction mixture was extracted with 20m*l* of chloroform (3 times), and the chloroform-soluble layer was collected, and dried over anhydrous sodium sulfate over night. The cloroform was removed by evaporation to dryness under the reduced pressure, yellow transparent oily subtance was obtained. Its was chromatographed on silica gel HF_{254} (thickness 0.5mm : 20×20cm) with a mixture of benzene-diethylamine (95 : 5). Under UV light, the major bands such as Rf-values of 0.57 (D), 0.36 (C), 0.23 (B) and 0.11 (A) were scraped respectively, and were extracted with chloroform (3 times) on glass-filter (15AG4,

Table 16. CP and its Metabolites in Chloroform-extracted Layer of ^{14}C taken up in the Larval Body of Rice Stem Borer through the Body of Rice Plant*

Time (days) after treatment	Radiocarbon Recovered as Indicated Product, %										
	Chloroform-extracted layer	Chloroform-soluble metabolites**									Water-extracted layer
		Origin	I	II	NF	III	DM	CT	IV	CP	V	
1 day after treatment	91.7	11.7	2.5	<0.1	7.1	<0.1	18.1	<0.1	<0.1	51.6	0.3	8.3
2 days after treatment	84.5	14.2	2.4	<0.1	7.1	<0.1	12.6	<0.1	<0.1	46.6	1.3	15.2
4 days after treatment	79.3	12.4	2.5	<0.1	7.2	<0.1	12.6	<0.1	<0.1	44.1	0.2	20.7
8 days after treatment	73.2	18.9	3.2	<0.1	7.3	<0.1	20.3	<0.1	<0.1	22.8	0.4	26.8

* the detail was partially referred to Table 15.

** the values of Rf were also same as that of Table 1.

made by Ohuchi Rika Manuf. Co., Ltd., Tokyo, Japan) and each chloroform-layer was evaporated under the reduced pressure. By co-chromatography and infrared absorption spectrum, the substance of Rf 0.57 (D) was identified to be non-reactive free *N*-(4-chloro-*o*-tolyl)-*N'*, *N'*-dimethylformamidine (CP). Also, the substance of Rf 0.23 (B) was found to be *N*-(4-chloro-*o*-tolyl-*N'*-methylformamidine (DM). The metabolite of Rf 0.11 (A) was identified as *N*-formyl-4-chloro-*o*-toluidine (NF). DM, extracted from reaction mixture, was treated with acetic anhydride and pyridine, and the molecular ion-peak (M^+) of the mass spectrum with *N*-(4-chloro-*o*-tolyl)-*N'*-acethyl-*N'*-methylformamidine. For CT (Rf: 0.23) recovered from the reaction mixture, although very samll amount, it was confirmed only with co-chromatography. Also, on the experiment *in vivo*, confirmation was done with co-chromatography.

DISCUSSION

CP was promptly metabolized in the *in vitro* experiment of rat liver-microsomes, giving DM and NF as the major metabolites. In this case, it was dependent on NADPH under aerobic conditions. This suggests oxidative metabolism in the microsomes+NADPH-system seemed to have involved. Moreover, the fact of involvement of the liver-microsomes system for drug metabolic enzyme was clear in view of the inhibition of the route from CP to DM by SKF-525A which is an inhibitor of O-, N-, and S-dealkylating enzyme (drugoxidizing enzyme). However N-methanol derivative which was thought to be an intermediate from CP to DM has not been detected. This might be attributed to unstability of the intermediate.

In incubating DM with rat liver-microsomes system, this system was also dependent on NADPH, and DM was metabolized and degradated into a compound such as NF showing lower insecticidal activities as described later. From the above results, the route from DM to NF was also dependent on rat liver microsomes+NADPH-system.

The formation of NF from CP seems to undergo by no enzymatic hydrolysis together with the enzymatic reaction, because considerable amount of NF was formed, even in the case of no addition of enzyme system (sucrose-buffer solution + NADPH). This enzyme system was dependent upon NADPH. However, the increasing rate of NF-formation due to the concentration of NADPH was slower than that of DM. And therefore, it must be ascertained physiologically whatever the hydrolytic enzyme system from CP to NF is due to NADPH as a cofactor.

The degradating route from NF to CT was not dependent on NADPH at the supernatant fraction, and considerable amount was degraded without the cofactor, therefore, it seems to suggest the involvement of formamidase which was located in supernatant fraction reported by Ahmad and Knowles (1971)[13,14].

The degradation of CT was dependent upon the microsomes + NADPH-system, and the existence of cofactor induced more numerous water-soluble product. The supernatant fraction was not related to the metabolism of CT with and without NADPH. CP showed rather slow metabolic speed quantitatively on the cellular fraction of whole larval body homogenate of rice stem borer, but its metabolic pattern seemed to be similar to that of the case in rat.

In vivo metabolic experiment in rat, CP was rather promptly absorbed, meta bolized and excreted.

In view of the components in urine, aqueous components were readily increased, reaching over 70% (male) and 80% (female) after 96 hours. As for the distribution of the components in chloroform-extracted layer of urine, DM was detected much within 24 hours, and the degradation from CP into DM was remarkable, showing the similar metabolic pattern to that in the case *in vitro*, this suggests that an activity of N-demethylase seems to be very high. Also, in time-course, CT which is the terminal metabolite in chloroform-extracted layer was increased but decreased after 48 hours, therefore, the route from CT to water-soluble products was considered. On the other hand, in the experiment *in vivo* on the larva of the rice stem borer, CP was very slowly metabolized quantitatively, but the metabolic pattern was the same. From the above results, the following major routes were estimated

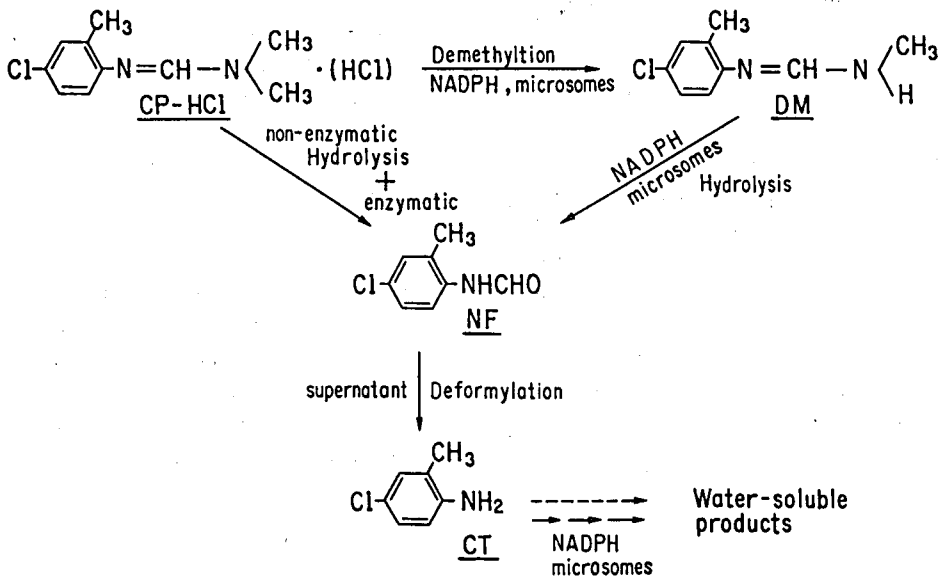

Fig. 5. Major pathways for chlordimeform-HCl metabolism on rat liver homogenate.

in summarizing the experiments *in vivo* and *in vitro* in rat (Fig. 5).

As for the biological activities of CP and its metabolites, the doses in oral administration to rat (mortality after 24 hours: ppm/mg) were CP, 270 ppm: DM, 264 ppm; NF, 560 ppm; and CT, 1,058 ppm[1,4], while in insecticidal experiment against the larva of the rice stem borer, 30 larvae were inoculated to each one stem of rice planted in the pot at seven leaf stage and each drug adjusted to 200 ppm 7 days after inoculation was sprayed with each 120 m*l* by the use of spray-gun (pressure: 2 atmospheric pressure) on the turntable, and after 7 days, the rice stem was peeled for investigating the survival or death of the larvae of rice stem borer, as a result, CP showed the mortality 100%, DM 85.0%, NF 0%, CT 20%[1].

From the above results, no higher active compound was observed than CP which is an original compound among the found metabolites, even in the metabolic experiments *in vivo* and *in vitro*. This effects showed that CP was gradually detoxicated in the metabolism of rat and rice stem borer larvae on both *in vitro* and *in vivo*.

The metabolic pattern of CP in the above two species of animals observed in the present experiment was similar even in higher animals such as dog, goat, and mouse[5,6], thus CP was proved to be well degraded. Whereas, in the insects, the experimental results on CP are very few[12], and as mentioned previously, CP in the larva of the borer was little degraded in both *in vivo* and *in vitro* showing similar metabolic pattern. Under the same reaction mixture as that at the liver of rat in the metabolic experiment *in vitro* on the larva of the borer, CP was very little degraded, and about 80% of the original agent of CP remained unchanged without being degraded. Also, its transference into water-layer was less than 1%, therefore, the detoxicating speed was very slow in the larva of the borer, as compared with that in the liver of rat.

Even in the test *in vivo*, the topical application of ^{14}C-CP-HCl to the larva of the borer resulted in showing the absorbed rate of 53.5% in the body after 24 hours, reaching 83.1% after 4 days. While, the sum of CP and DM which remained in the body after 4 days showed about 46.6% on the distribution of metabolites in the body, demonstrating the slow degradation. The penetration into the body was higher in the following

order, namely, at the epiderm, fat body, and digestive tube, and the metabolic pattern was nearly the same as the metabolic routes in higher animals, however, the degrading speed of CP was distinctly slow, thus rather highly effective amount of CP and DM remained in the larva of borer even after 4 days. With these results, mostly chloroform-extracted layer remained in high rate even on the change of uptake of CP in the larva of the borer through rice plant and also even in the rate between chloroform-extracted layer and water-extracted layer, namely, the rate was 73.2% even on the 8 days after treatment. As for the chloroform-extractable metabolites, CP, the original compound, showed the highest rate at all time in 1-4 days after treatment, and there was almost no change on the matabolic pattern, and the amount of CP showed about a half of total radioactive substances, followed by DM 10-20% showing insecticidal activities. Whereas, in rat after 4 days, over 90% of urinary radioactive substances were excreted, and only minor amount of CP and DM remained at the liver, kidney, heart, brain and blood plasma, therefore, there was distinct difference between the speed of metabolism, absorption and excretion of CP and the speed of degradation and detoxication of CP.

According to Ohsawa *et al.*[18], the result of the measurement of the content of P-450 and N-demethylating enzyme which are the drug-oxidizing enzyme on various living organisms showed 30 times higher content of P-450 in rat as compared with that in larva of the borer on rat liver-microsomes and microsomes of whole homogenate of larval bodies of the rice stem borer, also showed 6 time-higher activities on N-demethylase. The above fact showed the difference of N-demethylating reactivity as the difference of the function of biodegradation of CP between rat and the larva of borer.

In view of the above results, the differences of selective toxicity of CP against the rat and larva of the borer seems to be due to difference of enzymatic activities, namely the difference of N-demethylase, because of the clear quantitative difference of metabolism on the detoxication of the drug.

Moreover, another factor to control the selectivity is the difference of penetration, absorption and excretion of the CP between both species of animals besides the metabolic quantitative difference of metabolism.

As for the mode of action of CP, it had been reported that CP inhibited oxidative phosphorylation and stimulated ATPase in the cockroach mitochondria[19]. It also inhibited monoamine oxidase of mammalian liver[20,21] and cockroach head[22]. The synthesis of DNA, RNA and proteins were inhibited by CP[23]. More recently the effect of CP on the frog rectus adbominis muscle have been examined[24]. Chlordimeform (0.1-1 mM) suppressed the contracture induced by acetylcholine and potassium in a non-competitive manner. In a slightly higher range of concentration (1-10 mM), it caused a slow contracture. And also it had been concluded that block of neuromuscular transmission by CP is due primarily to the depression of the endplate sensitivity to the transmitter[25].

As the metabolites which showed higher biological activities than with the original compound in the metabolism of CP were not observed, the described results at this report must be a key to suggest the clarification of the mechanism of action of CP.

The non-competitive action on the contraction of rectus abdominis was recognized in CP, DM and Hokko 20013[21], all of which had the insecticidal activity, but no in NF and CT. Thus it had been found that there is a close relation between the non-competitive suppressive action on the contraction and the biological activities to insects and mammals by CP and its relative compounds (Ishibashi and Fukami, unpublished data).

And also present experiments seem to present a finding on the approach to the clarification of the selective reactivity of CP among various species of creature.

SUMMARY

The difference of action of chlordimeform [(*N*-(4-chloro-*o*-tolyl)-*N'*,*N'*-dimethylformamidine, CP)] against the rat and the rice stem borer seems to be due to the difference of metabolic

routes and degradation amount between the rat and the rice stem borer in view of the metabolic phase. On comparison between the biological activities of CP and its metabolites and each metabolic pattern, under the metabolic experiments *in vivo* and *in vitro* on rat and larva of the rice stem borer, rat showed the prompter absorption of ^{14}C-CP-HCl into the body and prompter excretion of ^{14}C-labelled radioactive compounds, and the degrading and detoxicating rates were also rather prompt. Especially, it was observed that the degrading rate from CP into *N*-(4-chloro-*o*-tolyl)-*N'*-methylformamidine(DM) and from *N*-formyl-4-chloro-*o*-toluidine (NF) to 4-chloro-*o*-toluidine (CT) and also from CT to water-soluble products was prompt and the enzymatic activities concerning these degradation was very high.

Whereas, in the larva of the rice stem borer, the absorption of ^{14}C-CP-HCl from the integument was slow, and the degradation and detoxication were very slow, therefore, CP and DM showing insecticidal activities were proved to be gradually accumulated in the body.

The metabolic pattern of CP was nearly the same as that in rat, therefore, the difference of metabolism, degradation and detoxication seems to be contributing to the selectivity of CP partially between the rat and the rice stem borer. ^{14}C-CP-HCl treated in water was permeated and transferred in the body of larva of the borer through the rice plant, being accumulated gradually, and metabolized partially.

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