DISTRIBUTION AND BILIARY EXCRETION OF CARBARYL, DIELDRIN AND PARAQUAT IN RATS: EFFECT OF DIETS

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Accepted May 24, 1980

Abstract—Carbaryl and dieldrin were accumulated mostly in the liver, and paraquat was accumulated in the kidney of normal diet (casein 24.5%) rats at 1 hr after the oral administration. At 5 hr after the administration, the concentration of carbaryl in liver or fat, and that of paraquat in kidney were markedly decreased. However, dieldrin in liver, kidney, lung, fat or others was significantly increased at the same period. The absorption of paraquat from gastrointestinal tract was the least among the chemicals used. Biliary excretion of carbaryl was the highest and that of paraquat was the least by 5 hr after intravenous administration. The excretion ratio was about 100:10:1 for carbaryl, dieldrin and paraquat in normal diet rats. Biliary excretion of these chemicals was higher in high protein diet (casein 45%) rats and retention of the chemicals in tissue was higher in low protein diet (casein 5%) rats.

Key words: distribution, biliary excretion, carbaryl, dieldrin, paraquat

INTRODUCTION

The steadily increasing use of chemicals in agriculture is known to cause environmental contamination and poisoning.

Man comes into contact with these chemicals via the skin, the respiratory system and the gastrointestinal tract. As the gastrointestinal route of exposure is particularly complex, we conducted experiments by the oral administration of common agricultural chemicals.

The aim of the present study is to characterize the effects of high- and low-protein diets on the distribution and biliary excretion of fat-soluble insecticides; carbaryl and dieldrin,
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and of water-soluble herbicide; paraquat, since nutritive value of diet, especially protein, seemed to have influence on the metabolism and probably the absorption of organic substances. Shakman (1974) has reported the influence of dietary factors on the toxicity of environmental pollutants.

Many organic chemicals are metabolized with microsomal enzymes in liver. Therefore, metabolism of chemicals will be influenced by protein diet. Studies on the absorption and metabolism of these chemicals have been reported by Dorough (1964), Heath (1964) Paulson (1970) and others. However, comparative studies among these chemicals have not been appeared in the literature.

MATERIALS AND METHODS

Four-weeks old male Spraque-Dawley rats were divided into 3 groups. Group 1 received a normal diet, group 2 a low protein diet and group 3 a high protein diet (Japan Clear, Ltd.) for 8–10 weeks, before experiments. The composition of diets is shown in Table 1.

Table 1. Diet composition

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Normal (group 1) (%)</th>
<th>Low protein (group 2) (%)</th>
<th>High protein (group 3) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>24.5</td>
<td>5.0</td>
<td>45.0</td>
</tr>
<tr>
<td>Corn starch</td>
<td>47.8</td>
<td>67.3</td>
<td>27.3</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>6.2</td>
<td>6.2</td>
<td>6.2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>9.5</td>
<td>9.5</td>
<td>9.5</td>
</tr>
</tbody>
</table>

14C-carbaryl (1-naphthyl n-methyl- 14C-carbamate), 14C-dieldrin and methyl 14C-parquat chloride (bis-n-14C-methyl-4, 4'-bipyridyl chloride) were obtained from The Radiochemical Center Amersham (USA).

Unlabeled carbaryl, dieldrin and paraquat (Wako Pure Chemical Industries, Ltd., Japan) were purchased commercially.

Concentration and radioactivity in the test solution was adjusted to 5 mg/10 μCi/ml corn oil for carbaryl, 0.46 mg/10 μCi/ml corn oil for dieldrin and 0.46 mg/10 μCi/ml water for paraquat by mixing radioactive and unlabeled chemicals.

Foods were withdrawn 16 hr before the experiment. The rats were lightly anesthetized with ether and administered with one ml of the test solution per kg body weight via a stomach catheter.

One or five hr after the administration, blood was collected exhaustively by heart puncture under ether anesthesia. The liver, kidney, spleen, brain, pancreas, lung, heart, testicles, abdominal fat, abdominal muscle and gastrointestinal tract were excised and...
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weighed. The gastrointestinal tract was separated into the stomach, duodenum plus jejunum, caecum and colon plus rectum, then rinsed with saline.

To determine biliary excretion, rats fasted for 16 hr were injected into the femoral vein with 0.2 ml of test solution per kg body weight under pentobarbital anesthesia (30 mg/kg i. p.), then bile was collected from polyethylene tube cannulated into the bile duct.

Approximately 250 mg of tissue samples, minced by scissors and mixed, were digested in 2 ml of NCS (Amersham-Searle, USA) at 55°C until completely solubilized. Rinsed saline of each separated gastrointestinal tract was diluted up to 50 ml with saline and 0.5 ml of the diluted rinsed saline was incubated with 2 ml NCS at 55°C for 30 min, respectively.

Blood samples (0.1 ml) were solubilized with 2 ml of mixture of Protosol (New England Nuclear, USA) and ethyl alcohol (volume ratio 1:2) at 55°C for 30 min, then 0.5 ml of hydrogen peroxide were added to decolorize. After the addition of 10 ml FCS (liquid scintillation cocktail, Amersham Corp., USA) to the solubilized samples, pH was neutralized with 0.5 ml of 2 N HCl.

After the four days of sample preparation procedure, ^14^C-radioactivity was determined in a liquid scintillation spectrometer (Mark II, Nuclear Chicago). ^14^C-radioactivity in the bile was determined in Mark II with Bray's scintillation liquid.

The radioactivity in gastrointestinal tract was calculated as a total of tissue and rinsed saline.

Statistical evaluations were made by using Student's test for significance at p<0.05 level.

RESULTS

Body weight and serum protein

Group 1 rats (normal diet) consumed approximately 15-23 g/rat of food per day, group 2 (low protein diet) consumed 7-13 g/day/rat and group 3 (high protein diet) consumed 11-17 g/day/rat. The average body weight of group 2 was significantly lower (p<0.01) than that of the other two groups throughout the 10 weeks observation period and there was no difference between group 1 and 3 (Fig. 1).

No difference of hematocrit levels was found in blood collected from the tail vein of three groups at 12th weeks after birth, while serum protein concentration of the same blood samples were significantly (p<0.01) different by dietary conditions. The protein concentration determined by refractometer (Hitachi) were 7.1±0.1, 6.0±0.1 and 8.2±0.1 g/dl in groups (six animals in each group) 1, 2 and 3, respectively.

Group 1

1. Distribution of radioactivity in tissue

Fig. 2 shows the radioactivity in tissue samples of rat at 1 hr and 5 hr after the oral administration of carbaryl, dieldrin or paraquat.

At 1 hr after the administration of carbaryl, radioactivity was highly detected in liver,
kidney and fat, then decrease was noted significantly at 5 hr after in liver and fat. By the administration of dieldrin, the highest radioactivity was detected in liver at 1 hr and increase was noted significantly in all tissues at 5 hr after. At 1 hr after the administration of paraquat, the highest radioactivity was detected in kidney and significant decrease was noted in liver, kidney, heart and testicles at 5 hr.

2. Radioactivity in the gastrointestinal tract

Radioactivity existed in the gastrointestinal tract, at 1 hr and 5 hr after the oral administration is showed in Table 2.
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Carbaryl

Dieldrin

Paraquat

Fig. 2. Distribution of $^{14}$C-radioactivity in tissues of rats fed with normal diet (group 1) at 1 hr (■) and 5 hr (□) after the oral administration of chemicals.

$10\mu$Ci/kg

Values are the mean±S.E. of three animals.

a: Significant difference between 1 hr and 5 hr (p<0.05)
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Table 2. "C-radioactivity* in the gastrointestinal tract of normal diet rat at 1 hr and 5 hr after the oral administration

<table>
<thead>
<tr>
<th></th>
<th>Carbaryl</th>
<th>Dieldrin</th>
<th>Paraquat</th>
<th>Carbaryl</th>
<th>Dieldrin</th>
<th>Paraquat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>26.36 ± 7.09</td>
<td>46.38 ± 3.22</td>
<td>20.03 ± 11.83</td>
<td>0.53 ± 0.44</td>
<td>6.05 ± 0.58</td>
<td>0.16 ± 0.09</td>
</tr>
<tr>
<td>Duodenum + Jejunum</td>
<td>26.67 ± 6.62</td>
<td>10.61 ± 2.81</td>
<td>74.83 ± 13.83</td>
<td>19.93 ± 1.10</td>
<td>18.93 ± 2.60</td>
<td>2.85 ± 0.35</td>
</tr>
<tr>
<td>Caecum</td>
<td>0.17 ± 0.04</td>
<td>0.17 ± 0.06</td>
<td>0.10 ± 0.03</td>
<td>12.13 ± 2.81</td>
<td>1.48 ± 0.66</td>
<td>59.90 ± 6.91</td>
</tr>
<tr>
<td>Colon + Rectum</td>
<td>0.10 ± 0.05</td>
<td>0.09 ± 0.01</td>
<td>0.23 ± 0.16</td>
<td>1.07 ± 0.93</td>
<td>0.92 ± 0.55</td>
<td>0.25 ± 0.11</td>
</tr>
<tr>
<td>Total</td>
<td>55.09 ± 1.17</td>
<td>95.50 ± 0.44</td>
<td>95.04 ± 2.05</td>
<td>32.48 ± 2.35</td>
<td>27.39 ± 1.48</td>
<td>63.28 ± 7.02</td>
</tr>
</tbody>
</table>

* % of dose (10μCi/kg)
Values are mean ± S. E. of three animals.

At 1 hr after the administration of three chemicals, radioactivity was detected mainly in the stomach and small intestine. At 5 hr, radioactivity in the stomach was highest when dieldrin was administered. Least radioactivity in the duodenum and jejunum was found when paraquat was administered. Highest or least radioactivity in the caecum was found when paraquat or dieldrin was administered. Total radioactivity existed in whole gastrointestinal tract was highest when paraquat was given.

3. Biliary excretion

Cumulative biliary excretion of "C-radioactivity in rats injected with chemicals into femoral vein is showed in Fig. 3.

The highest excretion rate in a period of first 5 hr was found in the carbaryl-injected rats; 15.5% of the injected dose was excreted during first 30 min and 27.3% had been excreted by 2 hr. Thereafter, the excretion curve kept the same level. In rats injected with dieldrin, approximately 0.1% of injected dose was excreted during the 30 min and then linearly increased upto 3.5% at the end of 5 hr observation period. The least amount of excretion was noted in paraquat-injected rats. At the end of 5 hr, only 0.3% of injected dose had been excreted.

Group 2 and Group 3
1. Distribution of radioactivity in tissue

Fig. 4 shows the radioactivity in tissue samples of rats fed on low protein (group 2) or high protein (group 3) diet.
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Fig. 3. Cumulative biliary excretion of $^{14}$C-radioactivity in rats fed on normal diet (group 1). The animals were injected with chemicals into the femoral vein.

*2μCi/kg

In group 2 rats administered with carbaryl, radioactivity in all tissues except kidney and muscle was significantly decreased at 5 hr after than 1 hr after the administration. In group 3 rats, administered with carbaryl, radioactivity in all tissues were similar at 1 hr and 5 hr after the administration. On the other hand, at 1 hr after the administration of carbaryl, the radioactivity in all tissues except muscle was significantly higher in group 2 than group 3, also radioactivity in kidney and brain, was significantly higher in group 2 than group 3 at 5 hr after.

When dieldrin was administered, radioactivity in all tissues except spleen of group 2 and all tissues of group 3 was significantly higher at 5 hr after the administration than at 1 hr. With dieldrin administration, radioactivity in spleen, brain and fat at 1 hr. and in pancreas, heart, fat and muscle at 5 hr was significantly higher in group 2 than group 3.

When paraquat was administered into group 2, there was only significant difference of radioactivity between 1 hr and 5 hr in kidney.

In group 3 administered with paraquat, radioactivity in liver, kidney, pancreas and muscle was higher at 1 hr than at 5 hr.

In rats paraquat administered, radioactivity in kidney and brain at 1 hr and in liver, kidney, spleen, brain, lung and testicles at 5 hr was significantly higher in group 2 than group 3.
Fig. 4. Distribution of ^14^C-radioactivity in tissues of rats fed with low protein diet (L, group 2) or high protein diet (H, group 3) at 1 hr (□) and 5 hr (▲) after the oral administration of chemicals.

*10μCi/kg

Values are mean±S. E. of three experiments.

a: Significant difference between 1 hr and 5 hr (p<0.05)

c: Significant difference between low protein diet rat and high protein diet rat at 1 hr (p<0.05)

e: Significant difference between low protein diet rat and high protein diet rat at 5 hr (p<0.05)
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2. Radioactivity in gastrointestinal tract

Radioactivity existed in gastrointestinal tract of group 2 and group 3 is showed in Table 3.

At 1 hr after the administration of three chemicals, significant difference of radioactivity between group 2 and group 3 was not observed in any part of gastrointestinal tract. At 5 hr after the administration of dieldrin, significant difference of radioactivity between group 2 and group 3 was noted in caecum. When paraquat was administered, there was a significant difference of radioactivity between group 2 and group 3 in duodenum plus jejunum and total of gastrointestinal tract.

3. Biliary excretion

Cumulative excretion of $^{14}$C-radioactivity in bile after the injection of chemicals into femoral vein of rats of group 2 and 3 are showed in Fig. 3.

Biliary excretion in group 3, irrespective of chemicals administered, was higher than that in group 2. In carbaryl-injected rats, the difference of biliary excretion between group 2 and 3 was observed immediately after injection. In dieldrin-injected rats, biliary excretion in group 3 was slightly higher than in group 2, and a difference of excretion rate appeared 2 hr after the injection in paraquat-injected rats.

**DISCUSSION**

Studies on the body distribution and biliary excretion of agricultural chemicals, carbaryl, dieldrin and paraquat in rats were performed under different dietary condition. Rats were orally administered with nontoxic dose of chemicals to know the absorptive and metabolic characteristics of each chemicals comparatively. Experiments were carried out at 1 hr and 5 hr after the administration because those characteristics seemed to appear more significantly when some chemicals remained in gastrointestinal tract and when those were continued to be absorbed. And characteristics of chemicals will be made clear by the collecting of data about body distribution, biliary excretion, absorptive property, blood concentration, affinity to tissue and others. Other data than those in the present paper will be described in the next paper.

By oral administration of the chemicals into normal diet rats, higher $^{14}$C-radioactivity was detected in tissues of carbaryl- or paraquat-administered rats at 1 hr after than at 5 hr after the administration while the result was opposite in tissues of dieldrin-administered rats (Fig. 2). This phenomenon would result from the difference in intestinal absorption, metabolism in liver, affinity to tissues and others.

Biliary excretion and distribution in liver at 1 hr after the administration were the highest with carbaryl among the three chemicals examined. However, reabsorption of carbaryl from small intestine was the least among these three chemicals (unpublished data). Dorough (1964) reported that carbaryl was converted by liver microsomes of rat to water-soluble metabolites. Therefore, it can be speculated that large part of
Table 3.  ‘C-radioactivity* in gastrointestinal tract of low protein diet rat (group 2) and high protein diet rat (group 3) at 1 hr and 5 hr after the oral administration

<table>
<thead>
<tr>
<th>Group</th>
<th>Carbaryl</th>
<th>Diethyl</th>
<th>Paraquat</th>
<th>Carbaryl</th>
<th>Diethyl</th>
<th>Paraquat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach 1 hr</td>
<td>33.26±3.91</td>
<td>25.47±11.02</td>
<td>30.45±7.66</td>
<td>0.19±0.05</td>
<td>4.97±0.51</td>
<td>2.23±1.89</td>
</tr>
<tr>
<td>Stomach 5 hr</td>
<td>39.22±7.66</td>
<td>77.15±22.85</td>
<td>44.90±10.18</td>
<td>1.17±0.68</td>
<td>6.29±4.06</td>
<td>0.19±0.02</td>
</tr>
<tr>
<td>Duodenum 1 hr</td>
<td>7.24±1.39</td>
<td>35.55±11.08</td>
<td>57.42±8.58</td>
<td>17.42±2.61</td>
<td>12.24±4.00</td>
<td>12.63±3.74*</td>
</tr>
<tr>
<td>Duodenum 5 hr</td>
<td>15.50±4.74</td>
<td>19.09±3.58</td>
<td>49.51±6.02</td>
<td>15.77±1.87</td>
<td>16.12±4.29</td>
<td>2.83±0.50*</td>
</tr>
<tr>
<td>Jejunum 1 hr</td>
<td>1.16±1.02</td>
<td>0.08±0.06</td>
<td>0.33±0.25</td>
<td>4.05±3.98</td>
<td>0.20±0.01*</td>
<td>36.09±10.18</td>
</tr>
<tr>
<td>Jejunum 5 hr</td>
<td>0.09±0.04</td>
<td>0.23±0.14</td>
<td>0.10±0.04</td>
<td>11.47±1.61</td>
<td>2.51±0.23*</td>
<td>57.97±8.86</td>
</tr>
<tr>
<td>Caecum 1 hr</td>
<td>0.21±0.08</td>
<td>0.15±0.14</td>
<td>0.12±0.06</td>
<td>0.07±0.03</td>
<td>0.11±0.03</td>
<td>0.29±0.18</td>
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<tr>
<td>Caecum 5 hr</td>
<td>0.04±0.02</td>
<td>0.16±0.04</td>
<td>0.10±0.03</td>
<td>2.30±0.87</td>
<td>9.53±9.15</td>
<td>6.91±6.17</td>
</tr>
<tr>
<td>Colon + Rectum</td>
<td>44.93±3.81</td>
<td>61.24±22.02</td>
<td>91.53±1.00</td>
<td>21.73±1.50</td>
<td>17.52±3.45</td>
<td>51.51±4.60*</td>
</tr>
<tr>
<td>Total 1 hr</td>
<td>54.87±9.43</td>
<td>88.58±11.41</td>
<td>92.43±4.73</td>
<td>30.71±3.09</td>
<td>34.45±9.62</td>
<td>67.90±2.50*</td>
</tr>
<tr>
<td>Total 5 hr</td>
<td>54.87±9.43</td>
<td>88.58±11.41</td>
<td>92.43±4.73</td>
<td>30.71±3.09</td>
<td>34.45±9.62</td>
<td>67.90±2.50*</td>
</tr>
</tbody>
</table>

* % of dose (10μCi/kg)
Values are the mean±S.E. of three animals.
* : Significant difference between group 2 and group 3 (p<0.05)

radioactivity existed in gastrointestinal tract of carbaryl-administered rats at 1 hr and especially at 5 hr, would be due to biliary excreta.

The toxic effect of carbamate is inhibition of cholinesterase activity (Cambon, 1979). The rapid recovery in man will be due to rapid metabolism of it in liver.

Gastrointestinal absorption of paraquat would be the least among these three chemicals because the highest radioactivity was retained in whole gastrointestinal tract and caecum, and the least excretion was observed in bile (Table 2). Paraquat absorbed from the gastrointestinal tract was mainly concentrated in kidney at 1 hr and significantly eliminated at 5 hr after the administration (Fig. 2). Renal damage, as reported by Lock (1979), would be suspected when high dose of paraquat was given.

By comparative study on low protein diet rats and high protein diet rats, interesting result was observed with carbaryl administration.

As showed in Fig. 4, in low protein diet rats orally administered with carbaryl, radioactivity at 1 hr was significantly higher than at 5 hr in all tissues, except kidney and muscle, but in high protein diet rats, such phenomenon was not observed.

Furthermore, radioactivity was more detected in tissues of low protein diet rats than in tissues of high protein diet rats. Probably, the difference between two groups of rats is mainly resulted from the higher metabolic function in liver of high protein diet rats,
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Fig. 5. Cumulative biliary excretion of $^{14}$C-radioactivity in low protein diet rat (group 2, L△△△) and high protein diet rat (group 3, H●●●).
The animals were injected with chemicals into femoral vein.
*2μCi/kg

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because radioactivity is rapidly excreted in bile of high protein diet rats than low protein diet rats after i. v. injection of carbaryl (Fig. 5) and large part of carbaryl absorbed is converted to glucuronide and sulfate metabolites in liver of rats (Knaak : 1965, Dorough ; 1964). Therefore it was assumed that at 1 hr after the oral administration, absorbed carbaryl was already cleared from tissues and excreted in bile of high protein diet rats.

Higher retention of radioactivity was showed in tissues of low protein diet rats rather than high protein diet rats especially in kidney of rats administered with carbaryl or paraquat and in fat of rats administered with dieldrin (Fig. 4). A possibility of higher toxic effect of the chemicals in these tissues of low protein diet rats will be expected.

Further researches about absorption of chemicals, under different protein diet conditions, are in progress in our laboratory.

ACKNOWLEDGEMENT

This study was supported by a grant from the Toyota Foundation (78-1-063).

REFERENCES