Effect of 2,4-Dichlorophenoxyacetic Acid on the In Vivo Metabolism of Acetate in Adult Rats

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More than 90% of the radioactivity was recovered in the expired CO_2, with small amounts in the urine and feces, from control adult rats receiving orally C^{14}-labeled acetate. Oral dosage of 2,4-D reduced the incorporation of acetate carbon into respired CO_2 and also affected its pattern of elimination. In control rats, two distinctive rates of $C^{14}O_2$ elimination were observed. At lower dosages, 10 to 20 mg. per kg. of body weight, the effect on $C^{14}O_2$ respiratory pattern was insignificant. At a dosage of 400 mg. of 2,4-D per kg. of body weight, this effect persisted for 48 hours. Total recovery of radioactivity during a 24-hour period was decreased as the dosage of 2,4-D increased, and, at a constant dosage, the total recovery was higher when the time between the administration of 2,4-D and labeled acetate increased.

A number of investigations have been carried out in an attempt to elucidate the effects of 2,4-D and related compounds on the various metabolic pathways in plants such as glucose metabolism (*3, 7, 14*), acetate metabolism (*8, 21*), protein synthesis (*9, 18*), or the change in chemical composition of the tissues (*20, 24*).

To date, several papers have been published concerning the toxicology of 2,4-D in sheep and cattle (*1, 5, 13, 17, 19*). There has been very little information concerning the metabolic effects of 2,4-D on mammals. The LD_{50} of 2,4-D for rats is 300 to 1000 mg. per kg. of body weight (*16*). Florsheim and Velcoff (*10*) have studied some of the effects of 2,4-D on the thyroid function of male rats. Using subcutaneous injections of sodium 2,4-D, they found tht 80 mg. per kg. per day did not affect thyroid, pituitary, adrenal, or testicular weight. However, when 100 mg. per kg. of 2,4-D was injected, both the thyroid and body weights decreased. In the lower doses of 2,4-D, there was an increase in iodine uptake by the thyroid. In work with rat liver mitochondria, Brody (*4*) has shown that 2,4-D is capable of uncoupling oxidation from phosphorylation. The present investigation was undertaken in an attempt to determine if 2,4-D would affect the metabolism of the metabolically important intermediate, acetate.

Materials and Methods

Adult white rats (5 to 9 months old) of an inbred Oregon State Wistar strain were used. The weights of female rats ranged from 250 to 280 grams, while the male rats weighed 350 to 400 grams.

2,4-Dichlorophenoxyacetic acid, 2,4-D, was obtained from Nutritional Biochemicals Corp. and was administered as the potassium salt. Crystalline sodium acetate-1-C^{14} and -2-C^{14} were obtained either from New England Nuclear Corp. or Volk Chemicals. Sodium acetate solution was prepared such that the specific activity was about 5×10^7 d.p.m. per ml. and had a concentration of 14.5 μmoles of acetate per ml.

The aqueous solutions of 2,4-D and sodium acetate were administered to the rats by means of a stomach tube. For the ease of handling, light anesthetization with ethyl ether was used before the dosing of male rats. No anesthetization was used for the dosing of female rats. After the acetate-C^{14} was administered, the rat was placed in a Delmar metabolism cage and the CO_2 was trapped by sodium hydroxide solution. The sodium hydroxide solution from the CO_2 trap was changed periodically and was analyzed for radioactivity after conversion of CO_2 to $BaCO_3$. The $BaCO_3$ was filtered onto a glass fiber disk, washed, and dried and the radioactivity counted with a thin mica window GM detector. All counts were corrected for self adsorption and background.

The urine samples were collected and clarified by centrifugation at low speed. Aliquots of 0.1 ml. were analyzed for radioactivity in a Packard Tricarb Model 314S liquid scintillation spectrometer. The radioactivity in the feces was obtained by extracting the feces with a sufficient volume of 50% ethanol. The solid materials were centrifuged out and an aliquot of the supernatant was analyzed for radioactivity. All countings were corrected for quenching by using C^{14}-benzoic acid as an internal standard.

Results and Discussion

Elimination Pattern of Radioactivity in Expired CO_2. Figure 1*A* shows a kinetic plot of the C^{14} expired by normal adult rats following oral administration of C^{14}-labeled acetate. There are two separate rates for the elimination of $C^{14}O_2$. The initial rate of elimination (1 to 8 hours postmedication) is very rapid and has a biological half-life of 4 to 6 hours, followed by a much

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slower rate of elimination (half life of about 25 hours). The appearance of two separate rates of $C^{14}O_2$ elimination represents two routes of metabolism of the acetate carbons. The initial rate of elimination is probably due to the direct reaction of acetate with CoA-SH, to form acetyl-CoA, which subsequently is oxidized to CO_2 through the TCA cycle. In the intact animal, the CO_2 would be present in the form of blood bicarbonate and as the blood bicarbonate is turned over, there is a subsequent release of CO_2 from the lungs. The slower, secondary rate of elimination is probably derived from acetate carbons which are incorporated into other metabolites, such as fatty acids and amino acids and are subsequently catabolized to CO_2. The maximum incorporation in the expired $C^{14}O_2$ appears in the first hour, indicating that the acetate molecule is absorbed quickly and metabolized to CO_2. This is in agreement with the report of others (*6, 12, 22, 23*). The rates of elimination for the methyl and carboxyl carbons are not exactly the same, suggesting that the two acetate carbons are not metabolized in quite the same manner. The $C^{14}O_2$ elimination rates of acetate-1-C^{14} in untreated adult rats are similar between the male and female animals (Figure 1*B*), even though the accumulative recovery of expired $C^{14}O_2$ was less in the male rat (Figure 1*A*). Figure 1, *C* and *D*, demonstrates the effect of 2,4-D on the $C^{14}O_2$ excretory patterns from acetate-1-C^{14} by a single female rat and a male rat, respectively. There are variations in the excretory patterns from individual 2,4-D treated rats, but the general pattern is always similar

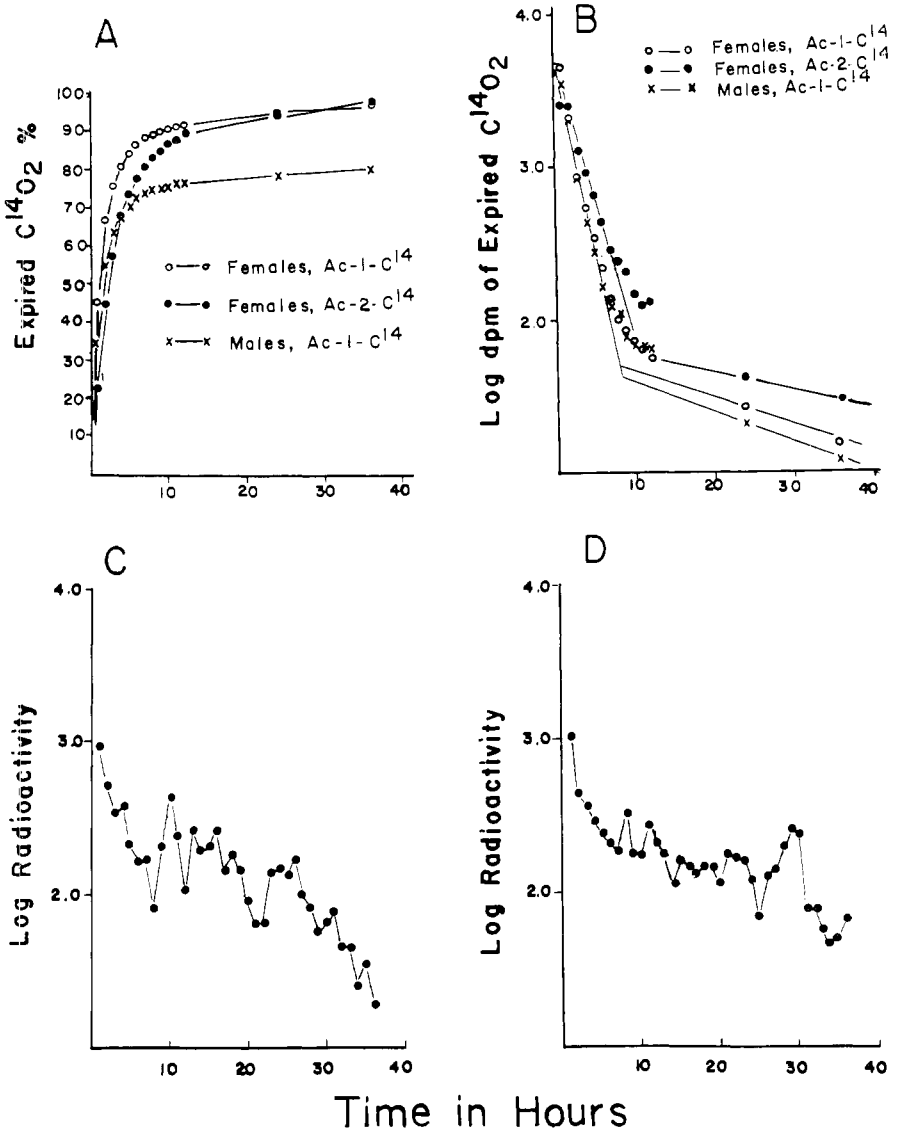

Figure 1. Recovery of radioactivity in the expired CO_2 from adult rats receiving C^{14}-labeled acetate

A. Accumulative recovery from normal adult rats. *B*. Kinetic plot of the expired $C^{14}O_2$ from normal adult rats. *C*. Kinetic plot of the expired $C^{14}O_2$ of an adult female rat treated with 400 mg. of 2,4-D per kg. of body weight and one hour later given acetate-1-C^{14}. *D*. Kinetic plot of the expired $C^{14}O_2$ of an adult male rat treated with 400 mg. of 2,4-D per kg. of body weight and one hour later given acetate-1-C^{14}

to the one presented. The initial rate of elimination is rapid, and is similar to that of untreated rats. However, after the first 6 to 8 hours, the rate changes, and appears unsteady. This fluctuation of $C^{14}O_2$ output remains during this 36-hour experimental period. This increase or decrease in the rate of $C^{14}O_2$ elimination pattern from acetate-2-C^{14} in female rats receiving 400 mg. per kg. of 2,4-D one hour prior to the administration of acetate-2-C^{14} is similar to those of acetate-1-C^{14} and is not present in the figure. The same was true for the male rats that recieved 400 mg. per kg. of 2,4-D and acetate-2-C^{14}.

The 2,4-D solution contained potassium phosphate, and to determine whether or not potassium phosphate may affect the $C^{14}O_2$ elimination pattern from labeled acetate, a dose of 400 mg. per kg. body weight K_3PO_4 in aqueous solution was given orally to female rats one hour prior to the administration of acetate-2-C^{14}. There was no difference in the elimination pattern from acetate-2-C^{14} between rats with or without receiving the potassium phosphate.

Route of Elimination. From 79 to 95% of orally administered acetate-C^{14} was recovered in the form of $C^{14}O_2$ (Table I). Only a small amount of the radioactivity was found in the urine and feces. These results agree well with those of others who administered labeled acetate either intravenously (*11*) or intraperitoneally (*2*). The radioactivity recovered from excreta during the first 24-hour period was considerably less from the males than from the females, either with or without 2,4-D treatment. Male rats apparently tend to fix more of the radioactivity in the body tissues, and the acetate carbons were not turned over as rapidly as in the females.

2,4-D treatment at 400 mg. per kg. of body weight dosage not only affected the pattern for $C^{14}O_2$ elimination but also reduced the recovery of the radioactivity in the expired $C^{14}O_2$. Even though the percentage of radioactivity in the untreated rats varies, the amount of radioactivity recovered under the influence of 2,4-D is about 70% of the control value (Table I *T/C* values). This inhibitory effect may have resulted from a reduction of acetate absorption in the gastrointestinal tracts, or of acetate turnover via the TCA cycle.

In Vivo Effect of 2,4-D on Acetate Metabolism in Adult Rats as a Function of Dosage. Because 400 mg. per kg. of 2,4-D affected the rate of $C^{14}O_2$ elimination, it is of importance to determine how small a 2,4-D dosage still will affect the metabolism of acetate. Two series of experiments which involved a total of 13 female rats were carried out using a 2,4-D dosage ranging from 10 to 600 mg. per kg. of body weight. The dose of 2,4-D was administered orally one hour prior to the administration of C^{14}-labeled acetate. Acetate-1-C^{14} was used in the first series and acetate-2-C^{14} in the second series. Figure 2, *A* and *B*, shows that 2,4-D had a pronounced effect on the $C^{14}O_2$ elimination pattern over a wide range of dosages. Dosages from 40 to 400 mg. per kg. of 2,4-D per rat appeared to have a similar effect on the $C^{14}O_2$ elimination pattern for both acetate-1-C^{14} and -2-C^{14}. Only at a dosage of 20 mg. per kg. of 2,4-D or less, did the effect on the elimination pattern seem to be decreased. The oral LD_{50} for 2,4-D in rats is 300 to 1000 mg. per kg. of body

Table I. Percentage of Administered Radioactivity Recovered in 24 Hours from Excreta of Adult Rats Treated or Not Treated with 400 Mg. per Kg. of Body Weight of 2,4-D and One Hour Later Given C^{14}-Labeled Acetate

	Females—Acetate-1-C^{14}			Females—Acetate-2-C^{14}			Males—Acetate-1-C^{14}		
	No 2,4-D	2,4-D Treated	*T/C*[a]	No 2,4-D	2,4-D Treated	*T/C*	No 2,4-D	2,4-D Treated	*T/C*
CO_2	95.3	66.5		89.4	63.5		79.2	55.9	
Urine	2.0	2.3		2.8	1.1		1.2	1.0	
Feces	0.5	0.1		0.5	0.7		0.3	0.1	
Total	97.8	68.9	70.5	92.7	63.5	70.5	80.7	57.0	70.6

[a] 2,4-D treated/control.

Table II. Percentage of Administered Radioactivity Recovered in 24 Hours from Excreta of Adult Female Rats Given Varying Doses of 2,4-D and One Hour Later Given C^{14}-Labeled Acetate

	2,4-D Dosage, Mg./Kg. of Body Weight							
	0	10	20	40	100	200	400	600
	Acetate-1-C^{14}							
CO_2	95.3		90.8	75.2	80.0	72.4	66.5	
Urine	2.0		2.4	0.9	2.6	1.7	2.3	
Feces	0.5		0.3	0.8	0.4	0.2	0.1	
Total	97.8		93.5	76.9	83.0	74.3	68.9	
	Acetate-2-C^{14}							
CO_2	89.4	86.6	77.6	60.9		62.5	63.5	53.4
Urine	2.8	2.8	2.4	1.1		1.2	1.1	1.9
Feces	0.5	0.3	0.7	0.4		0.4	0.7	0.4
Total	92.7	89.7	80.7	62.4		64.1	65.3	55.7

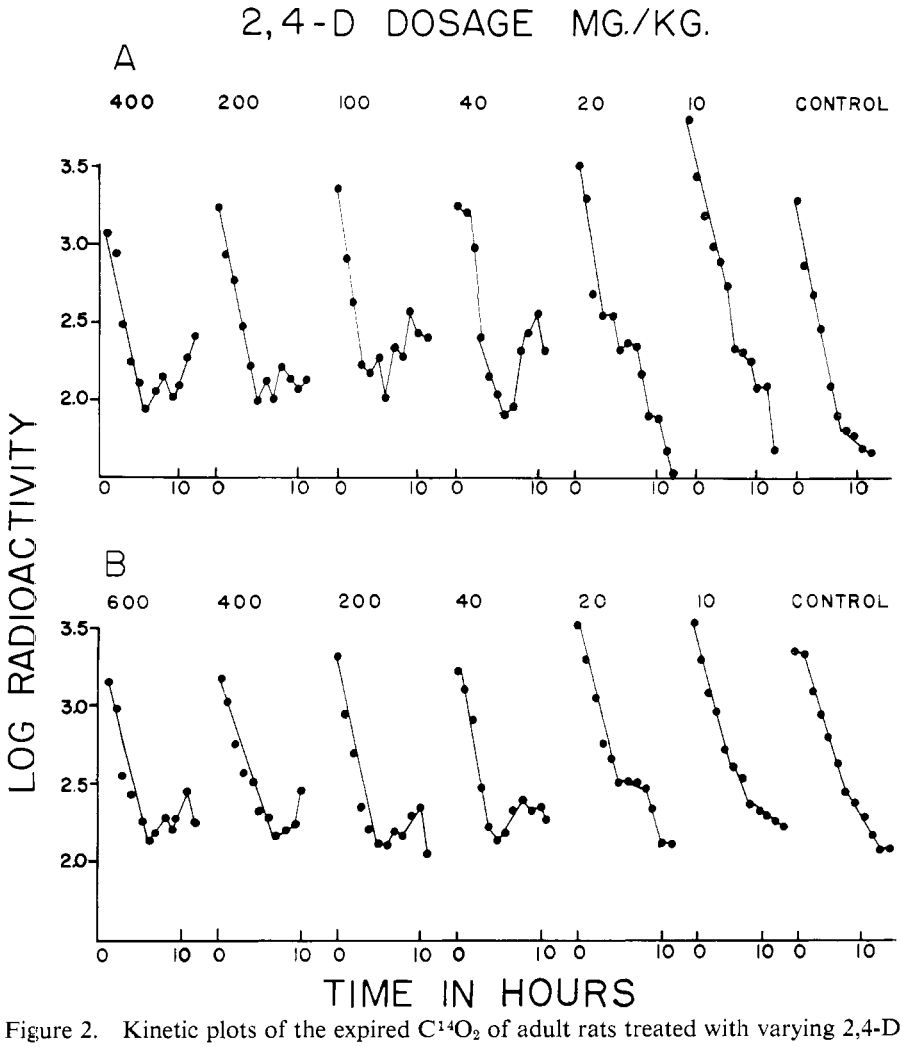

Figure 2. Kinetic plots of the expired $C^{14}O_2$ of adult rats treated with varying 2,4-D dosage one hour prior to the administration of C^{14}-labeled acetate

A. Acetate-1-C^{14}
B. Acetate-2-C^{14}

weight (*16*), and yet as little as 10 mg. per kg. of body weight still will alter the elimination pattern of $C^{14}O_2$ and the metabolism of acetate-2-C^{14}.

The recoveries of radioactivity in the excreta of adult female rats receiving acetate-C^{14} with or without 2,4-D are shown in Table II. It appears that 2,4-D dosages from 40 to 600 mg. per kg. of body weight definitely reduced the incorporation of radioactivity in the CO_2, and the degree of reduction appears to correlate with dosage. At 10 and 20 mg. per kg. of body weight dosages, the reduction is small and is comparable to that of the untreated one. Within experimental variation, the percentage of radioactivity recovered in the urine and feces is constant, and is not affected by 2,4-D over the entire range of doses tested. At every dosage, the percentage of radioactivity recovered from the acetate-2-C^{14} fed rats is consistently less than the percentage of radioactivity recovered from the acetate-1-C^{14} treated rats. This again reflects the observation that the carboxyl and methyl carbons of acetate are not metabolized in exactly the same manner.

Effect of 2,4-D on Acetate Metabolism in Adult Rats as a Function of Time after 2,4-D Treatment. Since the effect of 2,4-D on the in vivo metabolism of acetate-C^{14} was dependent upon the dosage of 2,4-D, and this effect from as little as 10 mg. per kg. of body weight of 2,4-D can be demonstrated from the $C^{14}O_2$ elimination pattern, the effect of varying the time between 2,4-D and acetate-C^{14} administration was investigated. Seven time intervals and a constant 400 mg. per kg. of body weight of 2,4-D were employed for this work. Figure 3 shows that the 2,4-D affected the metabolism of acetate, even when the 2,4-D was given 48 hours prior to the administration of the acetate. However, this effect disappeared completely after one week. This is significant in that although the effect of 2,4-D on acetate metabolism is dramatic for the short time intervals studied, the effect of 2,4-D

Table III. Percentage of Administered Radioactivity Recovered in Excreta of Adult Rats as Function of Time between Administration of 400 Mg. of 2,4-D per Kg. of Body Weight and Acetate-2-C^{14}

	Hours							
	1	2	12	24	36	48	168	No 2,4-D
CO_2	63.5	63.4	62.7	71.4	75.4	62.7	85.8	89.4
Urine	1.1	3.1	2.7	3.2	2.2	1.2	3.2	2.8
Feces	0.7	0.5	0.4	0.3	0.1	0.6	0.4	0.5
Total	65.3	67.0	65.8	74.9	77.7	64.5	89.4	92.7

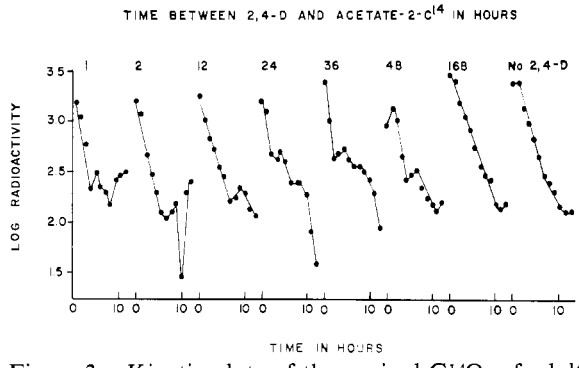

Figure 3. Kinetic plots of the expired $C^{14}O_2$ of adult female rats receiving 400 mg. of 2,4-D per kg. of body weight dosage and varying time later given acetate-2-C^{14}

was not long lasting; thus, an organism that received an oral dose of 2,4-D could be expected to recover in a relatively short period of time, if the dose of 2,4-D was not exceptionally large.

The recoveries of radioactivity in the CO_2, urine and feces, as per cent of administered acetate-2-C^{14}, for this series of experiments are shown in Table III. The percentage of radioactivity recovered appears to remain relatively constant from pretreatment of one hour through 48 hours and comparatively lower than that of the untreated control. However, at 168 hours, it is almost that found in the control value.

Recently, Khanna and Fang (*15*) have shown that 78% of an 80 mg. dose per rat of 2,4-D was excreted in the urine of rats within 48 hours, and the rate of 2,4-D elimination was dependent upon the dose of 2,4-D given. As the time between 2,4-D administration and acetate feeding is increased, the actual amount of 2,4-D in the tissues and organs should be decreased. Since the degree of effect is related to 2,4-D dosages, a progressive decrease in the effect of 2,4-D on acetate metabolism as the time increased could be explained on the basis of 2,4-D elimination. Since there was no observed effect of 2,4-D on acetate metabolism when the 2,4-D was given one week prior to the administration of acetate, it can be assumed that in a period of one week 2,4-D had been either removed from the site at which it affects the metabolism of acetate, or completely eliminated from the body. The method described in this paper should find useful application in the study of abnormal metabolic state as induced by toxic chemical in the intact animal.

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