Distribution and Excretion of Radioactivity in Rats After Oral Administration of H^3-3-Methylcholanthrene.* (25984)

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3-methylcholanthrene, a carcinogenic hydrocarbon of extremely high potency, will induce mammary cancer in susceptible strains of mice and rats when administered by mouth. Dose-response relationships, optimal conditions for tumor induction, and influence of hormones on tumor growth have been described for the rat by Huggins, *et al.*(1). According to these investigators, a single 10 mg dose induces mammary cancer in 20% of female Sprague-Dawley rats, age 50 days. The studies presented here were undertaken to acquire additional information regarding absorption, tissue localization (with particular reference to the mammary gland), and excretion of a carcinogenic dose of this hydrocarbon labeled with tritium.

Methods. Female Sprague-Dawley rats, age 50 days, were used. In the first of two experiments, concentration of tritium was determined in selected tissues, urine and feces of 3 rats 24 hours after administration of 10 mg H^3-3-methylcholanthrene by gastric tube. In the second experiment, daily excretion of tritium in urine and feces of each of 4 rats given a single 10 mg dose of H^3-3-methylcholanthrene by gastric tube was determined. H^3-3-methylcholanthrene, tritiated by the Wilzbach procedure(2)† and recrystallized to constant specific activity (2,000,000 dpm/mg disintegrations per minute) was dissolved in sesame oil to make a final concentration of 10 mg/ml. After administration of 1 ml of this solution, followed by 1 ml of sesame oil through a No. 8 French catheter, each animal was placed in an all-glass metabolism cage designed for complete and separate collection of urine and feces. On termination of the experiment, tissues for analysis were removed, weighed and minced with a fine scissors. Three 50-100 mg samples of each tissue were taken for analysis of tritium by the combustion method of Jacobson, *et al.*(3). The remainder of the sample was homogenized in distilled water and extracted 4 times with toluene. A 5 ml aliquot of the extract was added to 10 ml of "fluor"‡ solution and counted in a Packard Tricarb liquid scintillation spectrometer. This technic will recover 85% to 95% of known quantities of 3-methylcholanthrene added to tissue homogenates. The residue was dried *in vacuo* and analyzed by the combustion technic. Feces were dried in air, then ground to a fine powder in a mortar. Three 20 to 40 mg samples from each 24 hour collection were taken for analysis by the combustion technic. Each 24 hour urine sample was analyzed in triplicate by directly counting 0.3 ml in 4.8 ml of absolute ethanol and 10 ml of "fluor" solution. The efficiency of the counting system was determined by means of an internal standard, and all results were expressed as dpm.

TABLE I. Percentage Recovery of Tritium in Female Sprague-Dawley Rats 24 Hr after Administration of a Single Dose of 10 mg H^3-3-Methylcholanthrene by Gastric Tube.

Rat	1	2	3
Absorbed	6.5	5.6	5.3
Urine	4.9	4.4	3.9
Tissues	1.6	1.2	1.4
Unabsorbed	62.4	57.9	93.1
Stomach and contents	6.3	.2	31.9
Intestines and contents	32.1	37.0	52.8
Feces	24.0	20.7	8.4
Total	68.9	63.5	98.4

Results. Exp. 1. The percentage recovery of tritium 24 hours after administration of 10 mg H^3-3-methylcholanthrene is presented in Table I. Percentage of radioactivity recovered in tissues, urine and feces by the combustion technic was approximately 69 for rat

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† Dr. H. I. Jacobson and Dr. T. N. Jacobsen of Ben May Laboratory for Cancer Research prepared the tritium-labeled 3-methylcholanthrene used in these experiments.

‡ The "fluor" solution is prepared by dissolving 7.5 g diphenylaxazole, 0.075 g "POPOP" and 120 g naphthalene in 500 ml spectroscopic grade dioxane and 500 ml of redistilled xylene.

TABLE II. Total Radioactivity (dpm/g Wet Wt) in Tissues 24 Hr after Administration of a Single Dose of 10 mg H^3-3-Methylcholanthrene by Gastric Tube (Combustion Technic).

Rat No.	1	2	3	Mean ± S.E.
	× 1000			
Kidney	32.9	56	56.3	48.4 ± 7.71
Fat	31.6	24.6	20	25.4 ± 3.37
Liver	23.9	24.6	23.7	24.1 ± .28
Mammary gland	11.1	23.4	19.1	17.97 ± 2.20
Lung		15.1	9.1	12.1 ± .44
Uterus	11.3	7.1	17.4	11.9 ± 2.99
Brain		4.9	4.1	4.5
Skeletal muscle	2.2	2.5	3.3	2.6 ± .32
Blood	2.5			

#1, 64 for rat #2, and 98 for rat #3. Fifty-eight to 93% of the administered dose was recovered in gastric and intestinal contents and feces. It is assumed that these values represent, for the most part, material which has not been absorbed. Approximately 6% (6.5, 5.6 and 5.3, respectively) of administered radioactivity was found in the tissues analyzed, or in urine. Values obtained for urine (4.9, 4.4 and 3.9%, respectively) for the 3 animals were in good agreement.

Less than 2% of administered radioactivity was recovered from the tissues analyzed. The concentration per gram wet weight is presented in Table II for the combustion technic and in Table III for the toluene-extraction method. The results of the combustion method, which determines total radioactivity, were higher than those obtained by toluene extraction. This is to be expected inasmuch as the aqueous phase of the toluene-water system was not analyzed. The results presented in Table III represent only the concentrations found in the toluene extract and those in the dried residue.

With the exception of brain and skeletal muscle, all tissues analyzed contained at least 7000 dpm/g wet weight. Average concentration of total radioactivity ranged from 11,000 dpm/g for uterus to 48,000 for kidney. Mammary tissue and fat contained nearly comparable levels of total radioactivity; average concentrations were 18,000 and 25,000 dpm/g, respectively.

Sixty to 70% of the radioactive material in fat and breast tissue was toluene-soluble; less than 10% was extracted from the other tissues analyzed. These results suggest (1) that the radioactive material is in 2 (or more) chemical combinations, *i.e.*, one that is readily soluble in toluene and another which is insoluble, or (2) that the material is less firmly bound to adipose tissue.

Exp. 2. The daily excretion of tritium in urine and feces of 4 rats following a single feeding of 10 mg of H^3-3-methylcholanthrene is presented in Fig. 1 for urine and Table III for feces. Radioactivity was detected in urine of Rat No. 1 for 13 days, Rat No. 2 for 17 days, Rat No. 3 for 16 days, and Rat No. 4 for 15 days. With the exception of Rat No. 2 whose peak urinary excretion occurred on the second day, 3.5 to 4.0% of administered dose was excreted within the first 24 hours. These results are in agreement with those presented for Exp. 1. Detectable quantities (5 dpm/mg) of radioactive material appeared in feces of Rat No. 1 for 11 days, Rat No. 2 for 8 days, Rat No. 3 for 9 days and Rat No. 4 for 7 days. Of the original radioactivity administered, 71% for rat No. 1, 59% for Rat No. 2, 52% for Rat No. 3, and 45% for Rat No. 4 was recovered in feces during the first 5 days; trace amounts occurred intermittently throughout the remainder of observa-

TABLE III. Radioactivity in Toluene-Soluble Fraction and in Residue after Toluene Extraction, of Tissues from Sprague-Dawley Rats 24 Hours after Administration of 10 mg H^3-3-Methylcholanthrene by Gastric Tube.

	dpm/g wet wt					
	Rat 1		Rat 2		Rat 3	
	Extract	Residue	Extract	Residue	Extract	Residue
	× 1000					
Kidney	1.72	22.5	1.8	20.4	1.73	
Fat	20	.68	19.6	.48		
Liver	1.85	16.5	2.5	23	1.85	15.8
Mammary	11.5	2.77	13.5	.59	10.2	1
Lung			.66	9.5	.69	3.04
Brain			.4	2.4	.2	1.94
Skeletal muscle	.3	.824	.4	.63	.23	.9

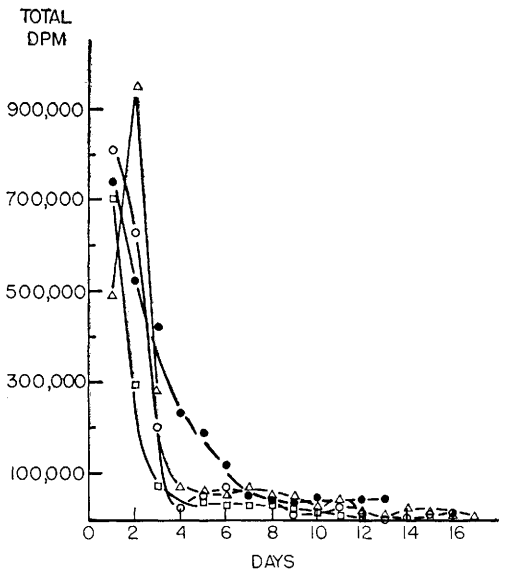

FIG. 1. Daily excretion of tritium in urine of 4 rats after administration of a single 10-mg dose of H^3-3-methylcholanthrene by gastric tube. 2000 dpm is equivalent to 1 μg of original hydrocarbon.

tion period (25 days). Trace quantities of radioactive material were found in mammary gland and liver but not in the other tissues from Rat No. 1 at 26 days.

Discussion. It is estimated that less than 10% of administered radioactivity was absorbed. The data obtained by toluene extraction are in good agreement with those of Dao, *et al.*(4), who analyzed a benzene extract of hydrolyzed tissue spectrofluorophotometrically. If the toluene-extractable radioactivity obtained in this study proves to be 3-methylcholanthrene, and on this point there is no information, concentration of the carcinogen per gram of tissue for the 3 rats is estimated to be 5.8, 6.8 and 5.1 μg for mammary gland, and 10 and 9.8 μg for fat, respectively. It would appear that this low concentration is sufficient to induce carcinogenesis in highly susceptible animals. This estimate is in agreement with observations in this laboratory that a single subcutaneous injection of 10 μg of 3-methylcholanthrene will induce sarcoma at site of injection in 2 of 10 rats.

Relatively high levels of tritium were recovered in all tissues except skeletal muscle and brain. Concentrations in kidney and liver were higher than those of mammary gland and fat. In contrast to fat and breast tissue, approximately 50% of total radioac-

TABLE IV. Excretion of Tritium in Feces after Administration of a Single Dose of 10 mg of H^3-3-Methylcholanthrene by Gastric Tube (Combustion Method), on 25 Successive Days.

Rat 1		Rat 2		Rat 3		Rat 4	
Total dpm (× 1000)	dpm/mg	Total dpm (× 1000)	dpm/mg	Total dpm (× 1000)	dpm/mg	Total dpm (× 1000)	dpm/mg
128	162	1,530	1,190	2,233	1,722	1,650	1,320
12,730	3,700	1,905.25	11,040	4,330	14,340	5,314	8,100
1,100	336	7,450	11,900	2,794	10,310	1,837	1,780
145	165	2,252.2	1,995	610.24	4,420		
102	147	648.8	406	477.32	2,010	312	172
15.4	32	93.23	81	1,635	694	86.6	36
21.13	21	230.55	56	897.6	548	12.81	8
5.44	16	9.914	6	191.17	122	2.4	1
3.04	8	4.57	1	51.82	20	.832	.3
12.04	7	3.14	1	4.85	2	2.2	1
6.5	5	1.38	1	.94	1	.0	0
2.65	3	2.99	2	2.46	1	1.54	1
.37	3	2.2	2	.505	2	.745	1
6.09	5	4.16	1	2.04	1	.177	1
3.08	3	1.1	1	.0	0	.24	.2
5.03	3	2.2	2	1.82	1	1.37	1
.42	1	2.59	1	3.36	1	1.415	1
1.69	2	1.53	1	.733	1	.68	1
4.89	1	1.15	1	6.4	3	6.36	5
3.64	2	1.3	2	.83	1	4.47	4
11	4	8.33	4	2.4	1	6.8	4
7.34	5	4.33	1	4.15	3	.99	1
16.6	6	1.9	1	.36	.3	2.77	1
8.25	4	2.364	2	.6	.3	1.67	1
11.48	9	.68	.4	.58	.3		

tivity was not extracted by water or toluene, but remained in the dried residue fraction. These results suggest that the administered hydrocarbon is rapidly converted to radioactive metabolites that are firmly bound to tissue. This type of protein binding has been established with other carcinogenic agents (5,6). Studies to determine the chemical nature of the binding of H^3-3-methylcholanthrene or its metabolites with tissue constituents are being undertaken.

The prolonged excretion of radioactive material in feces indicates that the carcinogen or its metabolites have a relatively long sojourn in the organism. The observations of Dao, *et al.*(4) and of Dauben and Mabee(7) support this interpretation. Failure to demonstrate significant quantities of radioactivity in tissues 25 days after administration of the hydrocarbon does not preclude its presence in trace amounts. It must be emphasized that the present technics for determination of tritium in biological material are not sensitive enough to detect with certainty concentrations below 5 dpm/mg. The small and variable quantities found in the feces after the first week may be due in part to the limitations inherent in the technic and in part to biological variation.

Summary. 1) Sprague-Dawley female rats, age 50 days, were given 10 mg H^3-3-methylcholanthrene dissolved in sesame oil by gastric tube. 2) In the first experiment selected tissues, urine and feces were analyzed for radioactivity after 24 hours. Sixty to 90% of administered radioactivity was found in gastric and intestinal contents or in feces; 4.4% in urine. Less than 2% was recovered in the tissues analyzed. Results obtained by combustion technic for measurement of total radioactivity were compared with those obtained by toluene extraction. Data indicate that most radioactivity in fat and mammary tissue could be extracted with toluene, whereas negligible quantities could be obtained by this method for liver, kidney, brain, uterus and skeletal muscle. 3) In the second experiment, 24-hour samples of urine and feces were analyzed for total radioactivity for 24-25 days. Significant quantities of tritium-labeled material appeared in urine for 13-17 days and in feces for 7-11 days. Thereafter, fecal and urinary excretion of radioactivity occurred intermittently in trace amounts.

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Action of Desoxycorticosterone Acetate* on Glycogen. I. Relationship of Liver Glycogen Formation to Potassium Metabolism. (25985)

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It has been established that a relationship exists between potassium and carbohydrate metabolism in the intact animal. Harrop and Benedict(1) were first to describe a decrease in blood serum potassium concentration following administration of insulin. They believed that this decrease was the result of potassium entering cells of tissue along with glucose during glycogenesis. This idea was supported by Fenn(2) who observed that potassium content of liver of intact rats approximately paralleled glycogen content. Gardner (3) reported that rats depleted of potassium

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