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The Distribution of Radioactivity in the Mouse Following Administration of Dibenzanthracene Labeled in the 9 and 10 Positions with Carbon Fourteen

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THE DISTRIBUTION OF RADIOACTIVITY IN THE MOUSE FOLLOWING
ADMINISTRATION OF DIBENZANTHRACENE LABELED IN THE
9 AND 10 POSITIONS WITH CARBON FOURTEEN

by

Charles Heidelberger and Hardin B. Jones

January 30, 1948

UNIVERSITY OF CALIFORNIA

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ABSTRACT

Health & Biology-General

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of Medical Physics of the Radiation
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30 January 1948

ABSTRACT.

Dibenzanthracene, labeled in the 9 and 10 positions with carbon fourteen has been administered to mice intravenously and by stomach tube as an aqueous colloid, and intraperitoneally, subcutaneously, and by stomach tube in tri-caprylin solution.

The distribution of radioactivity in the mice at various time intervals after administration of the carcinogen has been determined.

The radioactivity is rapidly eliminated, largely through the feces, and ordinarily very little is absorbed. The distribution and rate of elimination depends upon the mode of administration.

There is an appreciable quantity of radioactivity in tumors produced several months after a single subcutaneous injection of dibenzanthracene.

There appear to be no detectable effects from the radiation of the labeled carcinogen.

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January 30, 1948

Ever since the demonstration in 1930 by Kennaway and Hieger (2) that 1,2,5,6-dibenzanthracene, a pure chemical compound, is capable of producing cancer, there has been a great deal of interest and work in the field of chemical carcinogenesis. The isolation and characterization of 3,4-benzpyrene as one of the carcinogenic agents of coal tar by Hieger (3) and Cook and Hewett (4) in 1933 stands as one of the milestones in this field, and tied in the researches on occupational skin cancers with the ever increasing activity in the study of the carcinogenic hydrocarbons. This work was carried on chiefly by Cook and Kennaway in England and Fieser and Shear in America, and a very large number of related compounds were synthesized and tested for carcinogenic activity. As the synthetic activity was at its height, a series

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1. This paper is based on work performed under Contract #W-7405-Eng-48 with the Atomic Energy Commission in connection with the Radiation Laboratory, University of California, Berkeley, California. Preliminary reports of this work were presented at the meeting of the American Association for Cancer Research, Inc. in Chicago, May, 1947, at the Fourth International Cancer Congress, St. Louis, September, 1947, and at the 50th anniversary meeting of the Columbus Section of the American Chemical Society, October, 1947. Abstracts of these talks have been submitted as Reports BP-86 and BC-72 and have been declassified.
 2. E. L. Kennaway and I Hieger, Brit. Med. J. 1, 1044 (1930).
 3. I. Hieger, J. Chem. Soc., 395 (1933)
 4. J. W. Cook and C. L. Hewett, ibid 398 (1933).

of investigations were launched with the aim of elucidating the mode of action of these substances. This activity has mostly been concerned with the three compounds, 1,2,5,6-dibenzanthracene, 3,4-benzpyrene, and 20-methylcholanthrene, and centered about studies of the distribution, metabolism, and effect of these substances on the biochemical and physiological environment of the cell, epidermis, and the animal as a whole.

The analytical technique that has been employed in the researches on the distribution and metabolism of these carcinogens makes use of their characteristic fluorescence or absorption of ultraviolet light. It is this fluorescence that made possible the isolation of benzpyrene from coal tar. In the case of dibenzanthracene, the compound with which we are concerned in the present work, Berenblum and Kendall (5) and Chalmers (6) in 1934 found that after a few weeks, no carcinogen could be detected at the site of injection into the breast muscle of fowls. Hieger (7) in 1936 showed that dibenzanthracene administered subcutaneously to rats was retained for longer periods at the site of injection, whereas Berenblum and Kendall (8) demonstrated that there was a more rapid disappearance after intraperitoneal administration, and were unable to detect any carcinogen in the feces. Chalmers and Peacock (9) in the same year showed that a distinct fluorescence could be detected in the bile of mice following the intravenous injection of an aqueous colloid of methylcholanthrene and benzpyrene, whereas no such effect was observed with dibenzanthracene. Lorenz and Shear (10) were able to detect an appreciable quantity of dibenzanthracene in tumors pro-

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5. I. Berenblum and L. P. Kendall, Brit. J. Exp. Path. 15, 366 (1934).
 6. J. G. Chalmers, Biochem. J. 28, 1214 (1934).
 7. I. Hieger, Am. J. Cancer 28, 522 (1936).
 8. I. Berenblum and L. P. Kendall, Biochem. J. 30, 429 (1936).
 9. J. G. Chalmers and P. R. Peacock, Biochem. J. 30, 1424 (1936)
 10. E. Lorenz and M. J. Shear, Am. J. Cancer 28, 333 (1936).

duced six months after a single subcutaneous injection in lard.

In 1937, a significant advance in the knowledge of the metabolism of dibenzanthracene was made when Levi and Boyland (11) reported the isolation from rabbit excreta of a compound, which they characterized as being a dihydroxydibenzanthracene; in 1939 Dobriner, Rhoads, and Lavin (12) isolated a different dihydroxy compound from the urine and feces of rats and mice. This latter compound was shown by the synthesis of Cason and Fieser (13) to be 4', 8'-dihydroxydibenzanthracene. It is of interest to note that several phenolic metabolites of benzpyrene have been isolated and characterized.

R. N. Jones (14) and Jones, Dunlap, and Gogek (15) have made careful attempts, using ultraviolet spectroscopy as their analytical tool, to obtain a complete balance of elimination and retention of dibenzanthracene and its phenolic metabolite following subcutaneous and intraperitoneal administration. However, they were usually able to account for only about one third of the dose. Since only the intact pentacyclic aromatic ring system gives the characteristic fluorescence and ultraviolet absorption, it has been concluded by all workers in this field that there must be some more deep-seated degradation that occurs in the body that accounts for the complete disappearance of most of the carcinogen following various modes of administration.

With the availability from the U.S. Atomic Energy Commission of the long lived radioactive isotope of carbon, we decided to undertake a reinvestigation

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11. A. A. Levi and E. Boyland, Chem. and Industry 15, 446 (1937).
 12. K. Dobriner, C. P. Rhoads, and G. I. Lavin, Proc. Soc. Expt. Biol. N.Y. 41, 67 (1939); Cancer Research 4, 209 (1944).
 13. J. Cason and L. F. Fieser, J. Am. Chem. Soc. 62, 2681 (1940).
 14. R. N. Jones, Cancer Research 2, 237 (1942).
 15. R. N. Jones, C. E. Dunlap and C. J. Gogek, Cancer Research 4, 209 (1944).

of the distribution and metabolism of a carcinogenic hydrocarbon. It was hoped that a suitably labeled compound could be traced by following the radioactivity through the mouse, that this could be done with greater simplicity and accuracy than by the optical methods, that quantitative recoveries might be made, and that hitherto unknown metabolites might be discovered. All of these hopes have now been realized.

Dibenzanthracene was the compound chosen for this investigation, because a suitable synthesis was available, because a considerable body of data on its metabolic behavior was already in the literature, and because it is a symmetrical molecule that can be labeled in a symmetrical position, which might simplify the problem of identifying unknown metabolites. Accordingly, the synthesis of dibenzanthracene labeled in the 9 and 10 positions with carbon fourteen was carried out by Heidelberger, Brewer, and Dauben (16).

Apparatus and Methods

Each mouse was injected with a known amount of dibenzanthracene and was placed in a metabolism cage for the duration of the experiment. The cage was swept with a continuous stream of air which was bubbled through a tower filled with sodium hydroxide solution to trap expired carbon dioxide. Urine and feces were collected at periodic intervals, and at the appropriate time the mouse was sacrificed and dissected. The cage was washed with benzene and water. The specimens were kept frozen until assayed for radioactivity.

The respiratory carbon dioxide was precipitated as barium carbonate, filtered, dried, weighed, plated, and counted with a thin mica-window Geiger-Meuller counter according to the method of Dauben, Reid, and Yankwich (17). An aliquot of the urine specimens was plated and counted directly, and checked

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16. C. Heidelberger, P. Brewer, and W. G. Dauben, J. Am. Chem. Soc. 69, 1389 (1947).
 17. W. G. Dauben, J. C. Reid, and P. E. Yankwich, Anal. Chem. 19, 828 (1947).

occasionally by combustion. The feces and the tissue or organ samples were burned in oxygen in a standard organic combustion train. The carbon dioxide absorbed in sodium hydroxide, which was treated as described for the respiratory carbon dioxide. These samples were burned without drying in order to avoid the loss of volatile organic substances, and because under these conditions adequate sampling is impossible, the entire specimen was combusted in all cases. The total radioactivity of each sample was determined in this way, and was calculated as a percentage of the activity in the initial dose. The overall error in each determination is less than 5% and radioactivity corresponding to 1 gamma of dibenzanthracene can be determined with this accuracy. In those experiments where the total recovery of radioactivity is low, it is believed that the loss occurs by leakage from injection sites and from small accumulated errors in individual assays, rather than from gross errors or losses during the assays.

For these analyses we are deeply indebted to Mrs. Sally Brown, Mrs. Martha Kirk, Mrs. Mary Spilman, Mrs. Marion Perkins, Miss Mary Lee, Mrs. Beverly Dandliker, Mrs. Yvonne Stone, and Miss Martha Landefeld.

Distribution of Radioactivity Following Intravenous Injection

The dibenzanthracene was administered intravenously as an aqueous colloid, prepared as follows: 0.5 mg. of dibenzanthracene is dissolved in 0.3 ml. of redistilled acetone, and this solution is rapidly pipetted through a very fine orifice under the surface of one ml. of water in a centrifuge tube. A very finely dispersed colloid is formed, and the acetone is removed by bubbling a stream of air through the mixture, which is then made isotonic with 20 mg. of glucose. The colloid prepared in this way is usually stable for several days; one ml. is injected into the tail vein of each mouse.

TABLE I

	Experiment 3 A strain mouse 0 ⁺ wt. 17 g. 0.42 mg. colloid given intravenously 24 hours			Experiment 5 A strain mouse 0 ⁺ wt. 27.6 g. 0.341 mg. colloid given intravenously 48 hours		
	Wt. wet tissue	Total Counts	% of dose	Wt. wet tissue	Total Counts	% of dose
Dose		28,300			22,800	
Respiratory Carbon Dioxide	11.908	15	0.05	19.19	< 100	< 0.4
Urine		92	0.32		1,770	7.76
Feces		1,590	5.62		9,150	40.0
Gastrointestinal tract and contents	2.432	25,200	88.7	2.643	3,330	14.6
Liver	1.197	975	3.47	1.718	2,610	11.4
Salivary glands	0.078	73	0.26	0.053	196	0.86
Lymph nodes	0.017	0	0	0.105	96	0.42
Spleen	0.128	0	0	0.261	0	0
Seminal vesicles	0.278	0	0	0.159	0	0
Body fat	0.155	0	0	0.288	0	0
Skin and hair	2.792	0	0	3.274	0	0
Muscle	1.008	0	0	2.002	0	0
Bones	4.494	0	0	5.602	0	0
Brain	0.364	0	0	0.392	0	0
Lungs	0.101	104	0.37	0.155	87	0.38
Blood plasma	0.296	88	0.31	0.181	130	0.57
Blood cells	0.226	0	0	0.252	0	0
Kidneys	0.223	0	0	0.348	130	0.57
Heart	0.080	0	0	0.101	452	1.98
Mammary Carcinoma				2.767	1,180	4.90
Total Recovery		28,100	98.8		19,300	84.5

Table I summarizes the data obtained from mice sacrificed 24 and 48 hours after intravenous administration of the dibenzanthracene colloid. It will be noted that essentially a quantitative recovery of the dose has been found, in contrast to all previous work. This indicates that degradation products, if formed, are detected by their radioactivity, whereas fluorescence or ultraviolet absorption spectra are not adequate for this purpose.

There is an insignificant amount of radioactivity in the respiratory carbon dioxide; most of the activity is found either in the feces or the gastrointestinal tract. The urinary excretion is small compared with the amount found in the feces. In one mouse, a large mammary carcinoma already present took up a small amount of radioactivity, but this was considerably less than was found in the liver. However, it is evident that by this mode of administration there is little absorption of dibenzanthracene into the system, but that it is rapidly eliminated.

Table II shows that there is an extremely rapid initial uptake of the colloidal substance by the liver and the early appearance of activity in the intestinal contents. There is a measurable blood level in the early times, and

TABLE II
INITIAL STEPS IN DISTRIBUTION
0.5 mg. of aqueous DBA colloid injected intravenously

	% of dose		
	$\frac{1}{2}$ hr.	$1\frac{1}{2}$ hr.	24 hr.
Liver	89	82	3.5
Spleen	1.9	2.3	0
Plasma	0.24	0.10	0
Red cells	1.00	0.71	0
Intestinal contents and feces	1.9	7.6	94
Lungs	0.43	0	0.37
Total Recovery	94.7	97.0	98.8

the activity is more closely associated with the red cells than with the plasma, which may be a surface adsorption phenomenon since the material is in colloidal form. The presence of radioactivity in the lungs is in accord with the observations of Lorenz and Shimkin (18) made with methylcholanthrene, and which may explain the induction of pulmonary tumors following repeated intravenous injections of these carcinogens.

It seemed reasonable that the elimination must occur through the bile, although Chalmers and Peacock (9) failed to detect any fluorescence in the bile of mice after intravenous administration of dibenzanthracene colloids. A bile fistula was performed on a mouse in which a glass canula was inserted into the bile duct, which was then tied off so that all of the bile was removed and collected. Mice so treated live for about 24 hours, and Table III shows strikingly that fecal elimination following intravenous administration of dibenzanthracene colloid takes place exclusively through the bile.

TABLE III

BILE DATA FROM INTRAVENOUSLY INJECTED COLLOID
% Total Activity Given, 24 hours

	Normal Mouse	Bile Fistula Mouse
Feces	5.62	0
Stomach & Contents	4.15	0
Intestines	0	0
Intestinal Contents	48.30	0
Bile		53.2
Total	58.1	53.2

Distribution of Radioactivity Following Administration by Stomach Tube

Table IV shows the distribution of activity 24 and 48 hours after admini-

18. E. Lorenz and M. Shimkin, J. Nat. Cancer Inst., 2, 491 (1942).

TABLE IV

ADMINISTRATION BY STOMACH TUBE

	Experiment 7 A strain mouse O ⁺ wt. 28.5 0.517 mg. in tri- caprylin 24 hours			Experiment 29 A strain O ⁺ wt. 35 g. 0.452 mg. in tri- caprylin 48 hours			Experiment 8 A strain O ⁺ wt. 1813 g. 0.530 mg. in colloid 24 hours			Experiment 27 A mouse O ⁺ wt. 33.5 g. 0.380 mg. in colloid 48 hours		
	Wet weight	Counts	% of dose	Wet weight	Counts	% of dose	Wet weight	Counts	% of dose	Wet weight	Counts	% of dose
Dose		34,600			30,300			35,500			25,400	
Urine		556	1.66		1,310	4.32					580	2.3
Feces		13,800	39.9		22,500	74.4		17,100	48.8		20,600	81.0
Stomach and contents	2.490			0.376	79	0.26	0.299	5,360	15.1	0.449	220	0.9
Intestines		17,200		1.052	0	0	0.922	1,064	3.00	1.273	0	0
Intestinal contents			50.0	1.204	0	0	1.568	5,170	14.5	2.159	0	0
Liver	1.745	1,150	3.32	1.558	0	0	1.087	0	0	1.766	0	0
Recovery		32,700	94.9		23,900	79.0		28,700	81.4		21,400	84.2

stration of dibenzanthracene as an aqueous colloid and dissolved in tricapylin. Each mouse was dissected and burned completely as shown in Table I, but the only organs listed here are those containing significant amounts of activity.

It is plain that the radioactivity is eliminated almost exclusively in the feces so rapidly that there is no detectable activity in the intestines and intestinal contents 48 hours after administration. There seems to be little difference in the fate of the dibenzanthracene whether administered as a colloid or in tricapylin, and there is no evidence, aside from small amounts of activity in the liver and urines, that any appreciable quantity of material is absorbed into the body following this mode of administration.

Distribution of Radioactivity Following Intraperitoneal Injection

The results of a series of mice injected intraperitoneally with a solution of dibenzanthracene in tricapylin is shown in Table V. Again, each mouse was dissected completely, but only the organs containing significant amounts of radioactivity are listed. Figure I shows the rate of elimination of activity in the feces. The dotted line represents one mouse, and the solid line, another. The rates are entirely self-consistent for each individual mouse, but there is a difference in the elimination rates of the two mice. The dashed line represents the retention of radioactivity in the peritoneal cavity, and on this curve each point represents a different mouse, but again within the limits of individual variation, the results are consistent. This figure also shows that the activity disappears from the peritoneal cavity at a faster rate than it is eliminated in the feces.

A mouse with a bile fistula was given an intraperitoneal injection of dibenzanthracene, and Table VI shows that by this mode of administration there is a much smaller amount of activity found in the bile than by the intravenous

Table V

INTRAPERITONEAL INJECTION

Each mouse received 1.0 mg. of dibenzanthracene
in 0.30 cc. tricapylin

	% of dose					
	1½	1½	2	3	6	7 (days)
Total urine eliminated	0	0	1.26	0.85	1.14	3.9
Total feces eliminated	0	0	8.6	24.2	35.6	74.
Contents of peritoneal cavity	70.5	63.2	34.3	32.2	25.4	11.8
Stomach and contents	0.46	0.63	0.46	1.82	0.48	0.89
Intestines	1.77	2.9	2.9	4.04	6.9	5.6
Intestinal contents	5.45	7.5	5.1	6.9	2.6	0.85
Liver	0	2.5	2.2	3.9	5.5	4.5
Body fat	5.2	0.63	3.6	3.4	1.9	1.1
Kidneys	0.36	0.16	0.67	0.41	0.36	0
Carcass	0.24	0	0	5.4	7.4	9.3
Recovery of dose	86.	83.	70.	87.	100.	108.

TABLE VI

BILE DATA FROM INTRAPERITONEAL
INJECTION IN TRICAPRYLIN

	% Total activity given 18 hrs.
Feces	none
Stomach and contents	0.63
Intestines	2.9
Intestinal contents	7.5
Bile	0.36
	11.4

route, and this biliary elimination represents only a relatively unimportant proportion of the total fecal elimination.

Our data on retention in the body are in qualitative agreement with those of Berenblum and Kendall (8), but whereas they were unable to detect any fluorescence in the feces, we find that to be the principal route of elimination of radioactivity from the peritoneal cavity. The detection of activity in the carcass (a term which includes all parts of the mouse not otherwise specifically mentioned in Table V) is in qualitative agreement with the results of R. N. Jones, Dunlap, and Gogek (15), who found an appreciable quantity of dibenzanthracene in the carcass 14 days after intraperitoneal injection in tricaprylin.

A comparison of the intestines and the intestinal contents shows that at first there is more radioactivity in the contents than in the intestines themselves, whereas by the sixth day there is more activity in the intestines than in the contents. Since the weights involved are approximately the same,

the values of total radioactivity are roughly proportional to the specific activities. This observation suggests the possibility that the radiocarbon compounds are eliminated in the feces by direct passage across the intestinal walls, and that absorption into the rest of the body takes place through the intestines, but at present there is not sufficient evidence to permit any definite conclusions to be drawn about the actual mechanism of absorption or elimination following this mode of administration.

Retention of Radioactivity Following Subcutaneous Injection

There is considerable evidence that dibenzanthracene is retained for fairly long periods at the site of subcutaneous injection in fatty solvents. E. N. Jones (14) found 36% of the dibenzanthracene injected subcutaneously in olive oil in 8 weekly injections to two rats, and Hieger (7) showed qualitatively that dibenzanthracene administered in lard to rats was detectable for various periods of time after injection, depending on the dose. Berenblum and Shoental (19) have shown that the rate of elimination of benzpyrene from the mouse following a single subcutaneous injection in sesame oil is constant.

We have given a series of mice a single subcutaneous injection of dibenzanthracene in tricaprylin, have sacrificed them periodically, and the entire region of the site of injection was burned and assayed. The results showing the rate of disappearance from the site are shown in Figure II, and demonstrate a fairly rapid elimination during the first week, after which a constant slower rate is maintained. Each point of the curve represents a single mouse, and there is relatively little individual variation.

The feces of one mouse was collected during the first week following subcutaneous injection, and the results shown in Figure III indicate that again

19. I. Berenblum and R. Shoental, *Biochem. J.* 36, 92 (1942).

fecal elimination is the means of removal of dibenzanthracene from the mouse. There appears to be a more rapid elimination during the first two days, and then a steady rate is maintained. In this mouse 57% of the radioactivity remained at the site, and hence the total recovery in this experiment is 89%. This is in qualitative agreement with R. N. Jones (14) who has detected small amounts of dibenzanthracene in the feces of rats that had received massive subcutaneous doses of dibenzanthracene.

Retention of Radioactivity in Tumors Produced by Dibenzanthracene

Lorenz and Shear (10) in an important investigation showed that tumors produced 6-8 months after a subcutaneous injection of dibenzanthracene in lard still contained substantial quantities of dibenzanthracene.

To date, we have obtained six tumors that were produced by a single subcutaneous injection of the radiodibenzanthracene dissolved in either tricaprylin or mouse fat. These tumors were dissected when large, were homogenized, and an aliquot assayed for radioactivity. Table VII shows the results obtained.

TABLE VII
RADIOACTIVITY IN TUMORS PRODUCED BY DIBENZANTHRACENE

Time (months)	Solvent	Tumor	cts./min.	%
6	Mouse fat	Spindle cell sarcoma	1250	3.7
10	Mouse fat	Spindle cell sarcoma	980	2.9
4 $\frac{1}{2}$	Tricaprylin	Hyperkeratosis of the skin with early squamous-cell carcinoma	1460	5.75
6	Tricaprylin	Hyperkeratosis of skin with early subcutaneous spindle- cell sarcoma	745	1.2
6	Tricaprylin	Spindle-cell sarcoma	5700	9.25
8	Tricaprylin	Spindle-cell sarcoma with marked hyalinization	4000	7.8

There is considerable variation among individual mice, and the activity retained depends upon the solvent used, but in every case there is an appreciable amount of activity present. The lower of the values obtained from the tricaprylin experiments correspond fairly closely with those predicted by extrapolation of the curve in Figure II. The higher values do not seem beyond the range of individual variations that one might expect in that curve.

There is no evidence to suggest any difference between the rate of incidence of tumors in these experiments and in the controls, which were given equivalent doses of nonradioactive dibenzanthracene.

We are greatly indebted to Dr. Michael Shimkin and Dr. Isabella Perry for the histological examination of these tumors. They report that none are markedly different from the usual tumors induced by subcutaneous injection of dibenzanthracene (20).

Discussion of Results

In the foregoing discussion all results have been expressed as radioactivities. This radioactivity was introduced into the mouse in the 9 and 10 carbon atoms of dibenzanthracene, but the assays only show the presence of these labeled carbon atoms and give no information whatever as to the compounds into which they are incorporated. There is absolutely no justification to assume that the radioactivities obtained in this study represent unchanged dibenzanthracene, they merely show the presence of the carbon atoms originally situated in the carcinogen and may be in completely different compounds by the time the assays are made.

It must also be emphasized that our results are in no way contradictory to the earlier work done in this field. In these previous investigations the

20. M. Shimkin and W. K. Bryan, J. Nat. Cancer Inst. 4, 25 (1943).

only available analytical techniques were fluorescence and ultraviolet spectroscopy, which require the presence of the polycyclic hydrocarbon ring-system in essentially unaltered form. The very low quantitative recoveries of dibenzanthracene and dihydroxydibenzanthracene administered under a variety of conditions have always been correctly interpreted (5,6,7,8,9,12,14,15,19) as meaning that some deep-seated alteration of the molecule has taken place during its metabolic course. The fact that we obtain essentially quantitative recoveries of the administered dose does not in any way imply superior technique, but only that the analytical tool that we have used permits the tracing of any and all substances originally derived from the dibenzanthracene, also is capable of higher precision, and is free from such errors as those introduced by substances present in the tissues that are light-absorbing. The evidence for the metabolic degradation of dibenzanthracene, together with some of the chemical characteristics of these metabolites will be presented in the accompanying paper.

The absence of radioactivity in the carbon dioxide of respiration is clear proof that the dibenzanthracene is not metabolized in such a way that the 9 and 10 carbon atoms appear in any of the common biochemical intermediary metabolites (acetate, pyruvate, citrate, glucose, etc.), since these substances are known to be very rapidly oxidized; if they were present even in minute amounts, there would be an appreciable radioactivity in the respiratory carbon dioxide.

The results we have obtained show in general that the carcinogen is eliminated as rapidly from the body as each mode of administration will permit, and relatively little is absorbed into the body. The major route of elimination in all cases is through the feces, and urinary excretion is of relatively minor importance.

As nearly as can be deduced from evidence now at hand, the mouse treats the carcinogen as a toxic substance and handles it pretty much as would be physiologically expected depending on the mode of administration. In the process of elimination, the compound is drastically degraded, possibly as a detoxication mechanism. There is no apparent tendency for the substance to concentrate within a short period of time in neoplasms already present in the animal.

Although there are no noticeable effects from the radiation of the labeled carcinogen, it seemed desirable to attempt to calculate the radiation dose in an experiment. For this we have chosen the case of a subcutaneous injection in tricapyrin, where the substance remains at the same site over relatively long periods of time.

Calculate the radiation dose in the tissue immediately surrounding a subcutaneous injection of 1 mg. of dibenzanthracene in 0.3 cc. of tricapyrin.

$$\text{sp. act. of dibenzanthracene} = 67,000 \text{ cts/min/mg.} = 1.14 \times 10^6 \text{ dis/min}$$

$$\text{Average energy of } C^{14} \text{ beta} = .050 \text{ Mev}$$

$$\begin{aligned} 1.14 \times 10^6 \text{ dis/min} \times 5 \times 10^4 \text{ ev/dis} \times 1.6 \times 10^{-12} \text{ ergs/ev} \\ = 9.1 \times 10^{-2} \text{ ergs/min} \end{aligned}$$

$$1 \text{ day} = 1440 \text{ minutes,}$$

$$\text{So the intensity of radiation from the DBA is } 1.31 \times 10^2 \text{ ergs/day}$$

$$\text{Now there are .083 ergs/mg. of tissue/r}$$

$$r = 3 - \text{roentgen equivalent}$$

$$\text{So the total possible intensity of radiation} = \frac{1.3 \times 10^2}{.083} = 1570 \text{ r/day}$$

Consider the drop of tricapyrin subcutaneously as a sphere. Volume 0.3 cm^3

$$\text{Radius} = 0.42 \text{ cm.} \quad \text{Surface area} = 2.2 \text{ cm}^2$$

The $\frac{1}{2}$ absorption thickness of C^{14} = 0.0025 cm, and the $\frac{1}{2}$ range is 0.013 cm

Let us calculate the amount of radiation received by the tissue of $\frac{1}{2}$ thickness immediately surrounding the sphere of tricapyrin.

The effective radiation can only come from the depth of the range of the C^{14} beta ray, and under these conditions 90% of the radiation is lost by self-absorption, and hence 10% is effective.

$$0.420 - 0.026 = 0.394 = \text{radius of sphere} - \text{less whole range}$$

$$V = \pi^4/3 (0.394)^3 = 0.255 \text{ cm}^3$$

$$\text{Volume of irradiating segment} = 0.300 - 0.255 = 0.045 \text{ cm}^3$$

Surface area x $\frac{1}{2}$ thickness = $0.0055 \text{ cm}^3 = 5.5 \text{ mg. of tissue}$ immediately surrounding the sphere that is receiving $\frac{1}{2}$ of the radiation. $\frac{1}{2}$ is the directional factor.

$$\text{Dose} = \frac{1}{2} \frac{\text{intensity} \times \text{effective radiation} \times \text{irradiating volume}}{\text{wt. of tissue} \times \text{total volume}}$$

$$= \frac{1570 \times 0.1 \times 0.45}{5.5 \times .300 \times 2} = 2.1 \text{ r/day}$$

Thus the total irradiation received by the tissue immediately surrounding the droplet, as calculated from Figure 2.

1 day	2.1r
7 day	10.0r
35 day	47r
180 day	83r
(∞) "	84r

For comparison, in tracer experiments with radioactive phosphorus, a single mouse is regularly given doses of 16 r/day or 140 r in 20 days over the entire body, with no perceptible biochemical or other changes. If one considers the relative weights involved in the comparison, it will be seen that in the latter case the mouse is receiving, with no observable effects, a total irradiation of about 7000 times that of the dibenzanthracene mouse.

Summary

Dibenzanthracene, labeled in the 9 and 10 positions with carbon fourteen has been administered to mice intravenously and by stomach tube as an aqueous colloid, and intraperitoneally, subcutaneously, and by stomach tube in tri-caprylin solution.

The distribution of radioactivity in the mice at various time intervals after administration of the carcinogen has been determined.

The radioactivity is rapidly eliminated, largely through the feces, and ordinarily very little is absorbed. The distribution and rate of elimination depends upon the mode of administration.

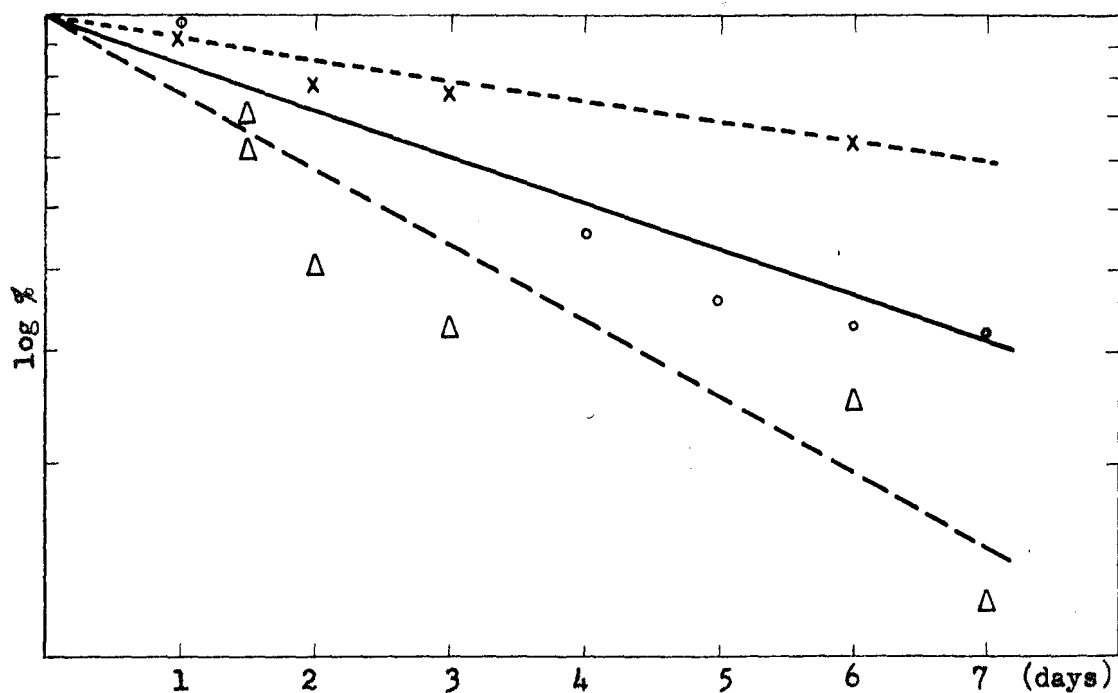
There is an appreciable quantity of radioactivity in tumors produced several months after a single subcutaneous injection of dibenzanthracene.

There appear to be no detectable effects from the radiation of the labeled carcinogen.

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Figure 1

Elimination in Mice Injected Intraperitoneally with 1 mg.
of Dibenzanthracene in Tricaprylin.



x--- Accumulated amount of elimination in feces, Mouse A

.___ Accumulated amount of elimination in feces, Mouse B

Δ ___ Retention in peritoneal cavity 6 mice

Figure 2

Rate of Disappearance from Site of Subcutaneous Injection in
Tricaprylin

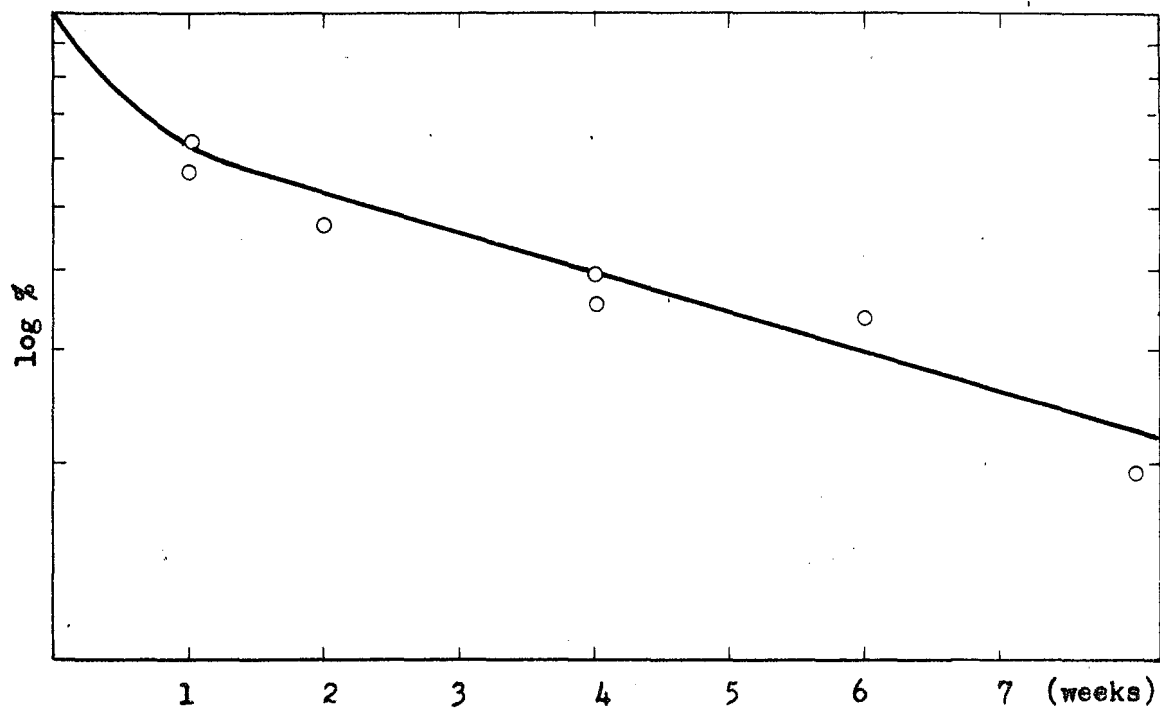
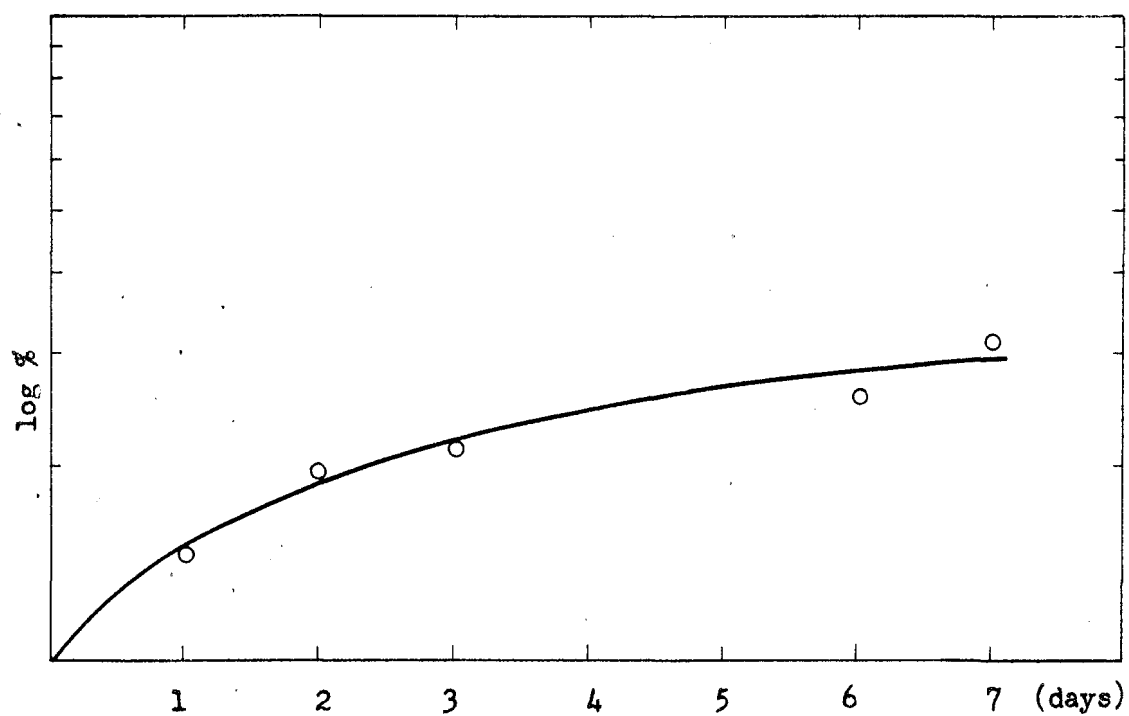


Figure 3

Accumulated Elimination in Feces from Subcutaneous Injection in
Tricaprylin



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