Fate of Subcutaneously Injected Benzo(rst)pentaphene in C57BL/6 Mice[1] (34699)

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Benzo [*rst*]pentaphene (more commonly called 3, 4, 9, 10-dibenzpyrene [DBP]) is an aromatic polycyclic hydrocarbon with carcinogenic potency (1, 2). When injected subcutaneously, 1 μg is sufficient to cause tumor production, and 50 μg will produce 100% tumor incidence (3). Early studies on this compound indicated that when injected subcutaneously it remains at the injection site and is not metabolized. Unseren and Fieser (4) were unable to detect either the compound itself or its possible oxidation products in extracts of urine or feces collected from hundreds of mice during 1 month following subcutaneous injection of 0.5 mg of DBP. However, the DBP could be extracted from the injection sites and from the tumor initiated by its action. The present report describes experiments designed to reinvestigate this problem by means of DBP tagged with carbon-14 (5). No difference in carcinogenic potency between labeled and unlabeled DBP was seen.

Materials and Methods. Fifty male mice of the C57BL/6 Jax line received subcutaneous injections of a 0.1 suspension of 500 μg of DBP in peanut oil (sp act 0.1 μC; 220,000 dpm). Groups of 5 mice were killed at biweekly intervals and the distribution of the radioactivity between injection sites and the following organs or tissues was determined: adipose tissue, adrenal glands, blood, brain, gallbladder, intestine, liver, lung, kidney, skeletal muscle, skin, spleen, thymus, and thyroid. Injection sites and the 14 organs or tissues from each animal were separately wet-ashed and the evolved CO_2 was trapped in barium hydroxide solution. The $BaCO_3$ precipitate was dried, finely ground, and suspended in 4% Cab-O-Sil (Cabot Co., Cambridge, Mass.) in diluted Liquifluor (Pilot Chemical Co., Watertown, Mass.). Radioactivity was then measured in a liquid scintillation counter. The overall recovery and efficiency of the system was 31%.

Results. Figure 1 shows the rate of disappearance of radioactivity from the injection site during the 20 weeks of the experiment. In 11 of the 50 mice, radioactivity was found in areas other than the injection site. The distribution of this radioactivity is listed in Table I. No radioactivity was found in adipose tissue, brain, blood, kidney, skeletal muscle, skin, spleen, thymus, or thyroid. There was no relationship between time and appearance of activity outside of the injection sites since radioactivity was found in

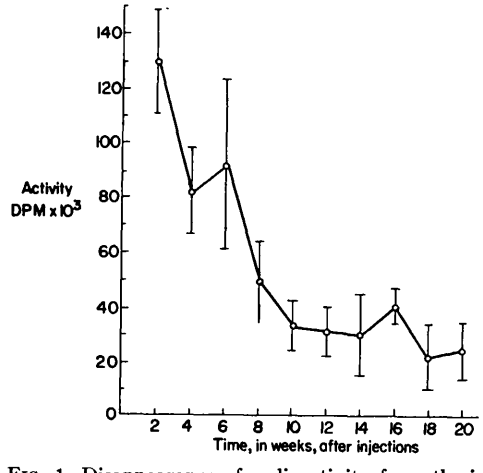

FIG. 1. Disappearance of radioactivity from the injection site; groups of 5 animals/point.

[1] Supported in part by U.S. Public Health Service Research Grant No. CA-04869 (National Cancer Institute) and General Research Support Grant No. SO1-FR-05525 (Division of Research Facilities and Resources), and in part by a grant from the Council for Tobacco Research-U.S.A.

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TABLE I. Distribution of Radioactivity in Areas Other than Injection Site in Mice Injected with ^{14}C DBP.

The 16 sites were found in 11 animals.

Location	No. of radioactive sites	Av activity (dpm)
Adrenal	3	9000
Gall bladder	1	50,000
Intestine	2	5500
Lung	9	54,000
Liver	1	3000

locations other than the injection site in 5 mice killed during the first 10 weeks and in 6 mice killed during the last 10 weeks of the experiment. In addition, there was no relationship between the rate of appearance of tumors and the amount of radioactivity found at the injection site. The average radioactivity at the injection sites of 16 tumor-bearing animals was 28,600 dpm, while 9 mice of similar age with no palpable tumors had average radioactivity of 31,800 dpm at their injection sites. Finally, there was no correlation between the amount of radioactive material lost from the injection site and the number or amounts of activity found in other sites.

Discussion. The mechanism accounting for the appearance of radioactivity in locations outside of the injection sites may be different for each location. Radioactivity in the intestinal tract is likely to have resulted from material which leaked from the injection wound and was ingested. The lung might show radioactivity as a result of suspended particles, or particles precipitated when the vehicle was metabolized and ingested by phagocytes which subsequently reached the lung. Radioactivity found in the adrenal gland may be related to mechanisms similar to those by which 7,12-dimethylbenzanthracene metabolites cause adrenal necrosis (6).

During the first 10 weeks, the amount of radioactivity at the injection site was reduced to about 15% of the injected dose. Thereafter, the level of radioactivity at the site was fairly constant. This may be due to the fact that the injected material by this time was encapsulated by connective tissue. The removal of DBP from the injection site may be a 2-stage process. First, since the amount of radioactivity and the number of radioactive locations outside of the injection sites did not increase with time, the removal of carcinogen from the original site must occur shortly after injection. This may be related to the trauma of injection. Secondly, the bulk of radioactivity is lost more slowly over a 10-week period and this material could not be detected. Since the nature of DBP would seem to preclude any metabolic degradation this removal must be a physical process, such as transport of small phagocytized particles, or more likely, dissolution of DBP in, or complex formation with, lipoproteins or proteins.

The ultimate fate of most of the compound after leaving the injection site is unknown. No radioactivity was found in exhaled CO_2. Random samples of urine and feces showed no detectable radioactivity which is in agreement with the work of Unseren and Fieser (4). It is possible that radioactivity due to DBP removal from the injection site was distributed through the body at levels below detection limits (2 $\times$ background) and excreted at the same levels.

Summary. Earlier studies indicated that the carcinogen, benzo[*rst*]pentaphene (more commonly called 3, 4, 9, 10-dibenzpyrene), remained at the site of subcutaneous injection. However, experiments with ^{14}C-labeled compound demonstrated that 85% of the carcinogen is removed from the injection site. This removal is accomplished in 2 stages: first, removal of significant quantities which can be detected at other body sites and which is associated with the trauma of the injection, and secondly, a chronic removal, nearly complete in 10 weeks. No relationship was found between the rate of tumor formation and the amount of carcinogen remaining at the injection site.

The author thanks H. Rys and A. Treger for technical assistance.

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Received Dec. 3, 1969. P.S.E.B.M., 1970, Vol. 133.