Distribution of Radioactivity in Plasma After Feeding Tritiated 7, 12-Dimethylbenz(a)anthracene.* (29983)

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The object of this study was to determine the uptake of tritiated 7, 12-dimethylbenz(a)-anthracene (DMBA-^{3}H) by the blood in the rat following oral administration of a single dose, and to assess its distribution in plasma proteins over a period thereafter. Initially DMBA-^{3}H was found in the neutral fat (chylomicra) and subsequently associated with the albumin fraction of plasma protein.

Materials and methods. DMBA-^{3}H, specific activity 800 μc per mg was produced by acid-exchange tritiation of highly purified 7, 12-dimethylbenz(a)anthracene (7, 12-DMBA) with tritiated trifluoroacetic acid (New England Nuclear Corp.) followed by chromatography on Florisil and recrystallization to constant specific activity. Tritium scan of a thin layer chromatogram of the product disclosed a single radioactive compound. This material was diluted with non-tritiated 7, 12-DMBA to a specific activity of 217 μc per mg and was dissolved in sesame oil to obtain a concentration of 15 mg per ml.

Female rats of the Sprague-Dawley strain, age 50 days weighing 150 $\pm$ 5 g were used. Five rats were each fed a single dose of 30 mg DMBA-^{3}H (6.50 mc) in 2 ml sesame oil by gastric intubation.

By cardiac paracentesis 0.2 ml of blood was drawn from unanesthetized rats at 2-hour intervals for the first 12 hours and at 24, 48 and 72 hours. From each sample of blood 0.1 ml was spotted on black Whatman filter paper and tritium activity determined after combustion in oxygen. The remaining blood was drawn into capillary tubes and centrifuged to determine hematocrit and to obtain plasma for further studies.

Five μl of plasma from each sample were analyzed by zone electrophoresis on 18 $\times$ 2.5 cm cellulose acetate membranes at 200 volts for 3 hours.‡ The strips were bisected longitudinally. One-half of each strip was stained with Ponceau S for densitometric evaluation of plasma protein fractions; the other half was preserved unstained for estimation of radioactivity. This latter portion was subdivided into 2 mm segments beginning 1 cm before the origin and extending to beyond the front zone as estimated by comparison with the stained strip. These segments were placed immediately into the counting vials each containing 5 ml of dioxane and left overnight to dissolve the cellulose acetate membrane. Next morning, 10 ml of scintillation solution§ were added and the activity determined in a Packard Tri-Carb liquid scintillation spectrometer.

Results. Uptake of DMBA-^{3}H by blood following oral administration. Tritium was observed in blood as soon as one hour after instillation of DMBA-^{3}H into the stomach (Fig. 1). There was a sharp rise in activity for 8 hours followed by a plateau for the next 4 hours. The activity then declined progressively over the next 60 hours. These results demonstrate rapid absorption of DMBA-^{3}H with maximal blood radioactivity levels between 8 and 12 hours; even 72 hours after administration significant quantities were still present. The initial hematocrit was 40%. Over the first 24 hours this had fallen steadily to 26% but by 48 and 72 hours the values had risen to 33 and

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‡ Electrophoresis was done in an LKB chamber. The stained strips were cleared by glacial acetic acid spray and placed between 2 layers of Dupont P40B Leader film, 35 mm. Densitometric evaluation of protein fractions was made on a Beckman Spinco Model RB Analytrol with Model R-102 microzone scanning attachment (B-2 balancing cam with 520 mμ front and 500 mμ rear interference filters).

§ The scintillation solution consisted of 240 g naphthalene; 150 mg 1,4-bis-2- (5-phenyloxazolyl)-benzene; 15 g 2,5-diphenyloxazole in 1 liter of 1:1 xylene and dioxane.

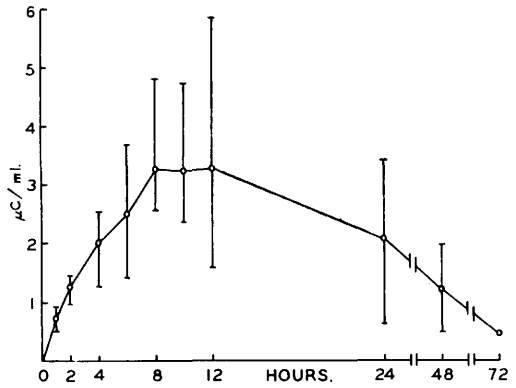

FIG. 1. Tritium activity in whole blood following oral administration of DMBA-^{3}H (6.5 mc) to 5 rats at 0 hr. Data represent mean values and range (μc per ml).

34%, indicating progressive hemodilution in response to the serial blood samples taken on the first day, with subsequent partial recovery. Although no determinations were done of the relative distribution of radioactivity between the plasma and the cellular components of the blood, these changes do not significantly influence the interpretation that maximal absorption of DMBA-^{3}H occurred between 8 and 12 hours.

Distribution of DMBA-^{3}H in plasma. There was a marked change in the distribution of tritium (Fig. 2-5) activity in plasma during the period from 2 hours to 48 hours after administration of DMBA-^{3}H. At 2 hours, 86.5% of the activity on the electrophoresis strip was in the region of the origin while only 9% had migrated with the albumin fraction. At 8 hours 37.5% and 28% of the plasma activity occurred in 2 maxima related to the origin and albumin zone respectively. At 24 hours these 2 fractions contained 20% and 31.3%, the remainder being distributed fairly evenly among the other protein fractions. At 48 hours 31% of the activity had the electrophoretic mobility of albumin.

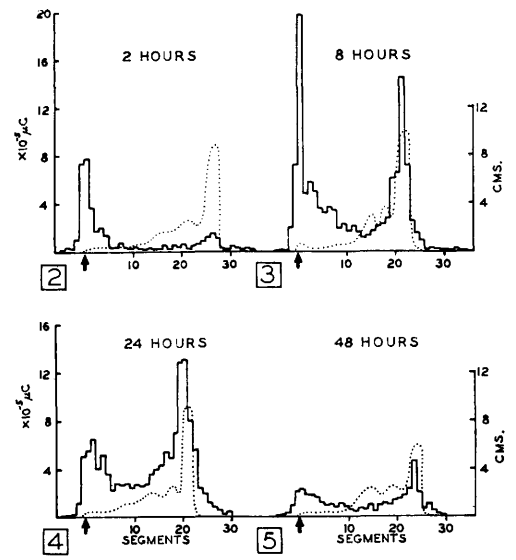

FIG. 2-5. Distribution of DMBA-^{3}H in plasma at 2, 8, 24 and 48 hr after oral administration. Histogram of tritium activity (———) is superimposed on protein electrophoretic patterns (·····). Arrow at origin.

Discussion. In the early hours after feeding DMBA-^{3}H the hydrocarbon is transported by that fraction of the plasma which remains near the origin on zone electrophoresis. It has been demonstrated by Swahn(1) that the constituents of this region are mainly neutral fats and chylomicra, and it is reasonable to assume that the hydrocarbon is absorbed in this lipide fraction since it was dissolved in sesame oil. Within a few hours, however, albumin assumes a major role in the transport of the tritiated material. Evidence indicating metabolism of the 7, 12-DMBA molecule by various tissues and organs in the rat has been reported by Flesher *et al* (2). The movement of radioactivity in plasma from the origin to albumin may reflect in part (a) metabolic transformations of the hydrocarbon to more polar substances which bind more tightly to protein and (b) decrease in plasma fat content with reduced capacity to retain compounds at the origin.

Of all carcinogenic hydrocarbons 7, 12-DMBA is the most powerful yet investigated. Its unique potential for induction of mammary tumors in Sprague-Dawley rats was clearly demonstrated by Huggins, Grand and Brillantes(3). Evidence has been produced by Huggins and Yang(4) that, among other features, the geometric resemblance of its molecule to steroids and to the purine-pyrimidine bases is of significance. There is a similarity in the transport of steroids and DMBA-^{3}H in plasma. Antoniades *et al*(5), using Cohn's cold ethanol Method 6, found that the proteins principally concerned in the transport of protein-bound steroids were those of

Fraction V (albumin) and of Fraction IV-I (α-globulin). The similarity of these results and those of the present study demonstrate yet another link between the properties of DMBA-^{3}H and those of steroids.

Summary. The appearance of tritiated 7, 12-dimethylbenz(a)anthracene in blood after oral administration to rats was determined and its distribution in plasma investigated. Initially the radioactivity was associated with neutral fat but after some hours the hydrocarbon migrated on electrophoresis with the albumin fraction.

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Effect of Prenatal Diet on Serum Cholesteryl Ester Fatty Acids in Newborn and Adult Rats.* (29984)

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Comparison of maternal and fetal plasma lipids has shown that in man and many other mammals the blood of the newborn contains lower concentrations of lipids than does the blood of the mother or the non-pregnant adult(1,2). Muldrey *et al*(3) found what appeared to be a characteristic "fetal" cholesteryl ester fatty acid (CEFA) pattern in human cord blood serum and in that of the newborn. This "fetal" serum CEFA pattern differs from what was then thought to be the typical "adult" serum pattern in that oleate was the predominant cholesteryl ester in fetal and neonatal blood, in contrast to the predominance of cholesteryl linoleate in adult blood serum. They reported that the concentrations of cholesteryl palmitate, oleate and arachidonate in cord serum were each approximately one-half of the corresponding values in the maternal serum, but that the cholesteryl linoleate concentration in cord serum was only one-tenth that of maternal serum. Thus, these differences must arise as the result of an "active process" which involves selective metabolic fates for the individual fatty acid esters of cholesterol. Zollner *et al*(4) have substantiated these findings of a human "fetal" and "adult" pattern of serum CEFA. A similar elevation of the cholesteryl oleate : linoleate ratio (O/L) in the serum of fetal and newborn animals of a number of species has been observed by Muldrey (unpublished observations). In the light of the evidence cited, experiments were designed in the present study to test the effect of certain variations in the fatty acid composition of prenatal diets on rat "fetal" and "adult" serum CEFA patterns and especially on the ratio of concentration of cholesteryl oleate : cholesteryl linoleate (O/L).

Furthermore, Okey and Lyman(5) suggested that a lipotropic effect of protein is most evident in a rapidly growing animal, in which a diet low in protein rapidly leads to fatty liver. Olson *et al*(6) showed that protein intake in man must be reduced to deficiency levels before the serum cholesterol level is depressed, and Moyer *et al*(7) reported similar findings in rats. Robinson and

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