acid mucopolysaccharide contents of the aorta, proliferation of the intima, and fragmentation or complete disappearance of the elastic tissue in the intima and media. The changes were most marked in the arch of aorta. Elastic tissue changes coupled with disturbances in the metabolism of mucopolysaccharides and collagen might alter the permeability of the arterial wall resulting in atheroma formation. The severity or degree of the different changes was neither related to the plasma level of cholesterol nor to the saturation or unsaturation of the vegetable oils used.

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Quantitative Estimation of 7,12-Dimethylbenz(a)Anthracene in Rat Mammary Tissue by Gas Liquid Chromatography.* (30383)

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The distribution of carcinogenic polycyclic hydrocarbons in animal tissues and their concentration at different intervals following administration have been studied mainly by spectrofluorometric measurements on tissue extracts(1-4). Autoradiographic techniques have also been used for study of the metabolism of radioactively-labelled hydrocarbons and their distribution in different organs of the rat(5,6).

In our laboratory, 7,12-dimethylbenz(a) anthracene (DMBA) is used for induction of mammary tumors in female rats by a single intragastric administration of the carcinogen dissolved in sesame oil, as advocated by Huggins and Yang(7). In connection with these

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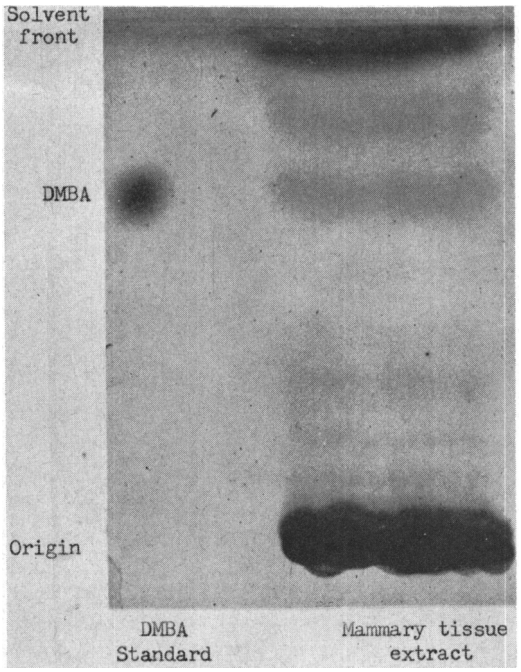

FIG. 1. Thin-layer chromatogram of an extract of mammary tissue from a rat treated with 20 mg DMBA and killed at 24 hr. Plate sprayed with conc. sulfuric acid and charred.

studies, a method utilizing a combination of thin layer chromatography (TLC) and gas liquid chromatography (GLC) has been developed for determining the concentration of DMBA in mammary tissue.

Experimental. Intact female rats of the Sprague-Dawley strain, 50-52 days old, were given a single dose of 20 mg DMBA in 1 ml sesame oil by intragastric intubation. They were then killed at various time intervals and each animal was skinned and the 4th pair of mammary glands resected and weighed. No attempt was made to separate glandular tissue from underlying mammary fat. Tissue from each animal was treated separately.

The tissue (0.5-1.0 g) was minced and hydrolyzed by refluxing for about one hour with 5 ml of 30% aqueous potassium hydroxide and 15 ml of absolute ethanol. After hydrolysis, the mixture was diluted with 10 ml of water, extracted twice with 20 ml of Skellysolve B (mainly hexane), and the combined extract was washed with 10 ml of water. For separation by TLC, the extract was concentrated to about 0.2 ml, applied in a band to a thin layer of Silica Gel G on a lantern slide plate ($3\frac{1}{4}'' \times 4''$) and chromatographed with Skellysolve B:ether (90:10 v/v) as developing solvent (Fig. 1). The DMBA was located by its fluorescence under ultraviolet light and the Silica Gel was scraped from this area and eluted 3 times with 2 ml portions of warm Skellysolve B. A DMBA standard was run on each plate so that the proper area for scraping could be located even when DMBA levels in tissue extracts were too low to give visible fluorescence. DMBA standards gave a single spot on the chromatograms when freshly recrystallized from methanol-water but a non-fluorescent impurity with lower R_f formed gradually on storage. Polynuclear hydrocarbons are susceptible to photodecomposition(8) and tissue extracts were therefore exposed to light as little as possible and the use of UV light was kept to a minimum.

An aliquot of the material eluted from the TLC plate was analyzed by GLC on a Barber-Colman model 10, using an ionization detector with a radium source (Fig. 2). The column ($6' \times \frac{1}{8}''$) was packed with siliconized Gas Chrom P coated with 3% SE-30 and was operated at 200°C with an argon flow rate of about 100 ml/min. The amount of DMBA was calculated by comparing the peak area with that obtained by injecting a known amount of DMBA standard. The product of peak height and retention time was used as an estimate of peak area(9). The limit of sensitivity of the method was about 0.05 μg DMBA. Experiments in which 20 μg quantities of DMBA in 100 μl of sesame oil were added to breast tissue and carried through the entire analytical procedure gave an average recovery of 95.5% $\pm$ 5.9 for 9 determinations.

Fig. 3 represents the concentration of DMBA in mammary tissue at intervals of 3 to 120 hours after its administration. The level rose sharply, reaching a peak at 6 hours, then declined until at 120 hours it averaged 0.2 μg/g of mammary tissue. Extracts of mammary tissue from untreated rats showed no peak corresponding to DMBA when analyzed by GLC.

Discussion. In the analysis of DMBA in

rat breast tissue by GLC, hydrolysis of the tissue was a necessary first step in order to separate the non-saponifiable fraction, containing the DMBA, from the large amount of triglyceride fat. The hydrolysis mixture used in our experiments was modified from that of Dao *et al*(1) which in our hands failed to give complete hydrolysis, even after 6 to 8 hours.

Thin-layer chromatography of the non-saponifiable fraction was used to separate DMBA from material which otherwise interfered with GLC estimation (Fig. 1). Extracts from DMBA-treated rats showed a fluorescent band at the same R_f as the DMBA standard, varying in intensity with the level of DMBA in the tissue. Another band of fluorescence was usually seen near the origin, but material eluted from this area showed no DMBA peak on GLC.

Systematic studies of DMBA concentration in breast tissue following its intragastric administration have not been made previously, but Bock and Dao(2) reported an average concentration of 23.4 μg/g breast tissue 24 hours after intragastric administration of 30 mg DMBA. In our studies, a 20 mg dose of DMBA gave a peak level of 16 μg/g after 6 hours (Fig. 3). Experiments with 3-methylcholanthrene in other laboratories(1,4) have shown that the time course of its accumulation and subsequent loss from mammary tissue is similar to that observed for DMBA.

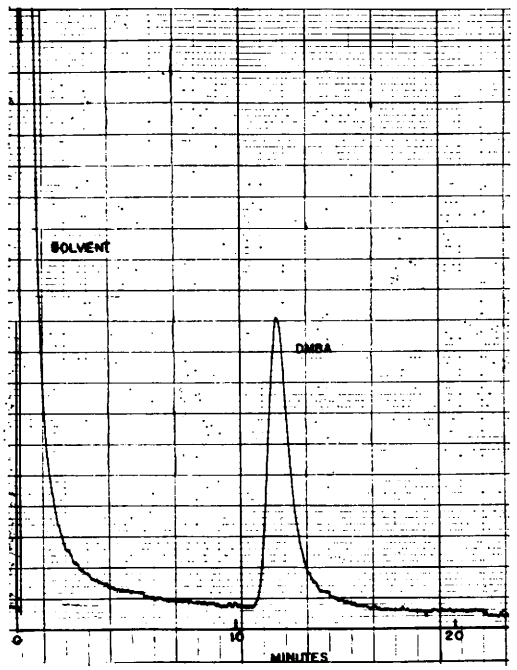

FIG. 2. Gas-liquid chromatogram of material eluted from a thin-layer plate in area corresponding to DMBA. Original extract prepared from mammary tissue of a DMBA-treated rat. Peak shown is equivalent to 0.36 μg DMBA. Chromatographic conditions as described in text.

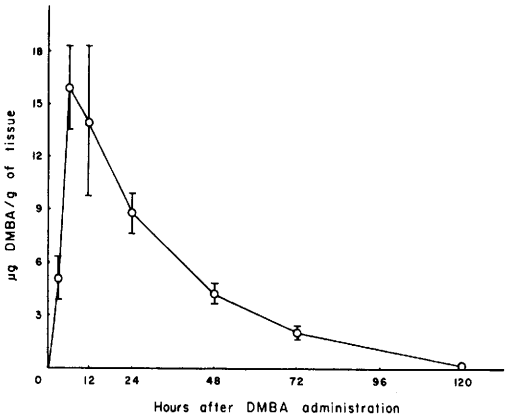

FIG. 3. Concentration of DMBA in rat breast tissue at different time intervals after administration of 20 mg DMBA in 1 ml sesame oil. Each point represents an average of 4 to 7 determinations and length of vertical bar represents Standard Error of Mean.

Preliminary experiments have shown that the present method could also be used for measuring 3-methylcholanthrene in mammary tissue. Under the conditions described, it migrated with DMBA on thin-layer plates but was readily separated by GLC, having a retention time 1.6 times that of DMBA.

Summary. A combination of thin-layer and gas-liquid chromatography was used to determine the concentration of 7,12-dimethylbenz(a)anthracene in mammary tissue of female rats at various intervals after its intragastric administration. Peak concentration was reached after 6 hours and measurable amounts were still present after 5 days. The method is as sensitive as spectrofluorometry, offers greater specificity and is applicable to the determination of other polynuclear hydrocarbons.

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Studies of Trace Metal Metabolism: Electron Paramagnetic Resonance of Manganese in Ribonucleic Acids.* (30384)

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In previous investigations(1-4), attention has been focused upon the subcellular metabolism of nickel and other trace metals, with especial emphasis upon the binding of trace metals to ribonucleic acids. These studies have confirmed the findings of Wacker and Vallee(5) that preparations of RNA from diverse biologic sources contain trace metals, including chromium, manganese, iron, nickel, copper and zinc. The investigations of Wacker and associates(5-10) have indicated that intramolecular metal bonds constitute a tertiary structure of RNA which stabilizes the helical secondary structure in a manner similar to the stabilization of proteins by disulfide bridges. The ligand interactions of the trace metals in RNA have not been established, although it has been suggested that the metals may be bound either to phosphate groups or to nitrogen atoms of the purine and pyrimidine bases(5,9-12).

In the present investigation, measurements have been made of the electron paramagnetic resonance (EPR) spectrums of RNA isolated from human and rat tissues. Principal consideration has been directed to the ligand interactions of manganese, since the EPR spectrum of manganese has been identified in samples of RNA(12,13), and since information is available regarding the interpretation of paramagnetic resonance patterns of manganese in a wide variety of compounds(14, 15).

The biological applications of magnetic resonance spectroscopy have been reviewed by Ingram(16), and by Jardetzky and Jardetzky(17). Determinations of the electron paramagnetic resonance of an atom are based upon changes in the absorption of microwave radiation which are induced by variations in the strength of a magnetic field. The EPR spectrum is sensitive to the electronic environment of the atom, *e.g.*, the way in which an atom is attached within a particular molecule, or the relationship of an atom to its surroundings within a crystal. Because of this sensitivity, measurements of electron paramagnetic resonance provide a means for the investigation of ligand interactions.

Methods. Preparation and characterization of RNA. Samples of RNA were prepared from human and rat tissues by a phenol extraction procedure(1,18). The samples of RNA were placed in cellophane sacs and dialyzed for 48 hours at 4°C against a volume of 5 liters of water, which was changed 6 times. RNA was precipitated with cold ethanol and dried in a vacuum desiccator over phosphorus pentoxide for 48 hours at 25°C. Immediately prior to spectrographic analyses or measurements of electron paramagnetic resonance, the samples were again dried *in vacuo* for 48 hours. Spectrographic analyses of trace metals were performed by the arc technique as previously described(1). Ultra-

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