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Organ-Specific DNA Damage Induced in Mice by the Organotropic Carcinogens 4-Nitroquinoline 1-Oxide and Dimethylnitrosamine (38938)

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The precise role of DNA damage and DNA repair in chemical carcinogenesis remains unknown (1–3). Many chemical carcinogens, including 4-nitroquinoline 1-oxide (4NQO) (4) and dimethylnitrosamine (DMN) (5), show varying degrees of organ specificity with regard to tumor induction. In order to correlate these observations with levels of DNA damage in various tissues, we have employed the biophysical technique of velocity sedimentation of DNA, in alkaline sucrose gradients (ASG), released from mouse lung, kidney, and liver preparations following treatment *in vivo* with various doses of 4NQO and DMN.

The ASG technique has been applied to monitor shifts in DNA fragmentation patterns of liver DNA from rats treated with chemical carcinogens (6–9). Few investigators have applied the technique to study DNA fragmentation in other organs (10, 11). Among the several factors required to control the sedimentation behaviour of DNA in alkaline sucrose gradients (12), a gentle controlled method of tissue manipulation and extraction of DNA destined for gradient analysis is crucial (13, 7). In this paper, we report a novel technique whereby lung tissue can be prepared for ASG analysis in a manner designed to reduce mechanical shearing of constituent DNA. More meaningful comparisons can therefore be made with the results of ASG analyses of liver and kidney DNA damage.

Material and Methods. For labeling of DNA, infant Swiss mice were injected sc with [H]thymidine (sp act 20 Ci/mmol; New England Nuclear) beginning 2 days after birth and continung every second day for a total of seven injections of 25 μCi each injection (25 μl). Mice were used when 4–6 mo old. For protein labeling, the method of Cox *et al.* (7) was modified. Mice (4–6 mo old) were injected with 0.1 ml (25 μCi) ^{14}C-labeled amino acid mixture (New England Nuclear) every 4 hr for a total of 100 μCi/animal given in four injections. Mice were used 4–6 hr after the last injection.

Chemical carcinogens were administered sc: 4NQO and 4-amino-quinoline 1-oxide (gifts from Dr. Y. Kawazoe, National Cancer Centre Research Institute, Tokyo) in dimethylsulfoxide (DMSO) vehicle (0.1 m/25 g body wt); DMN (K and K Laboratories, Plainview, NY) in 0.9% sodium chloride (0.1 ml/25 g body wt).

Following treatment with chemical carcinogens or solvent vehicle alone (controls), mice were killed by cervical dislocation and exsanguinated. Liver, kidneys, and lungs were removed quickly and placed in EDTA/saline buffer. Liver and kidney cell suspensions were prepared by the method of Cox *et al.* (7), with all operations conducted at 4° until gradients were loaded at room temperature. Cell concentrations were estimated in a hemacytometer chamber. About 10–50 μl of cell suspension, containing about 1 × 10^5 cells, were routinely layered.

Lung tissue preparations were conducted in a large plastic culture dish (Falcon Plastics) over crushed ice. Tissue pieces were manipulated with watchmaker tweezers. A 1–2 mg (wet weight) piece of lung tissue was selected at random from a lung lobe, placed in a dry area of the culture dish and minced in 10–15 μl of added EDTA/saline buffer (4°) with a cold, sharp razor blade until all pieces were sufficiently fragmented to pass through the bore of a 10-μl Corning disposable microsampling pipette (e.g., Cat.

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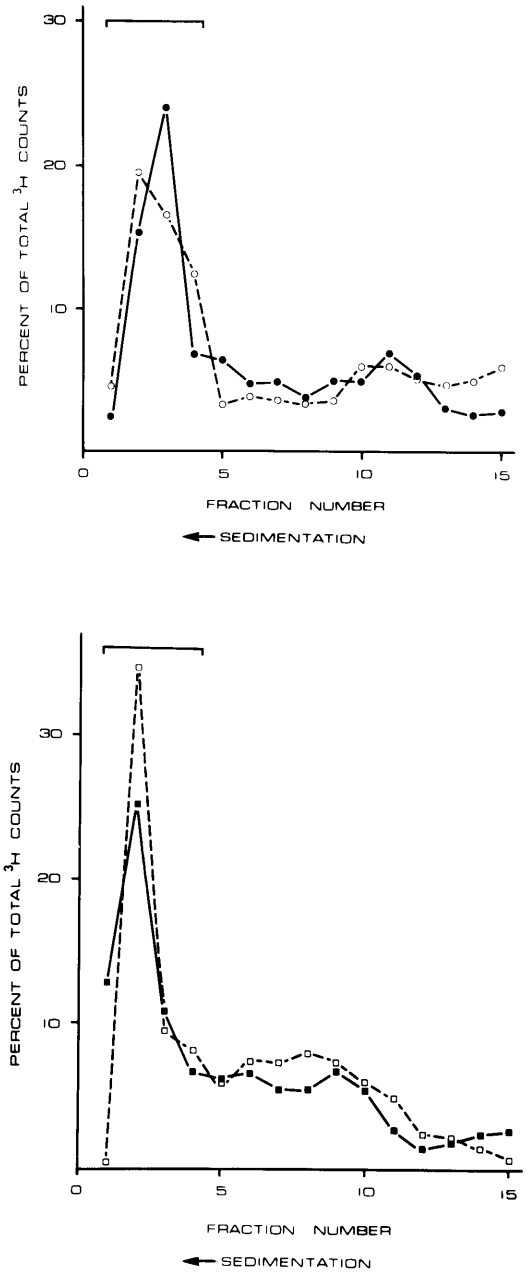


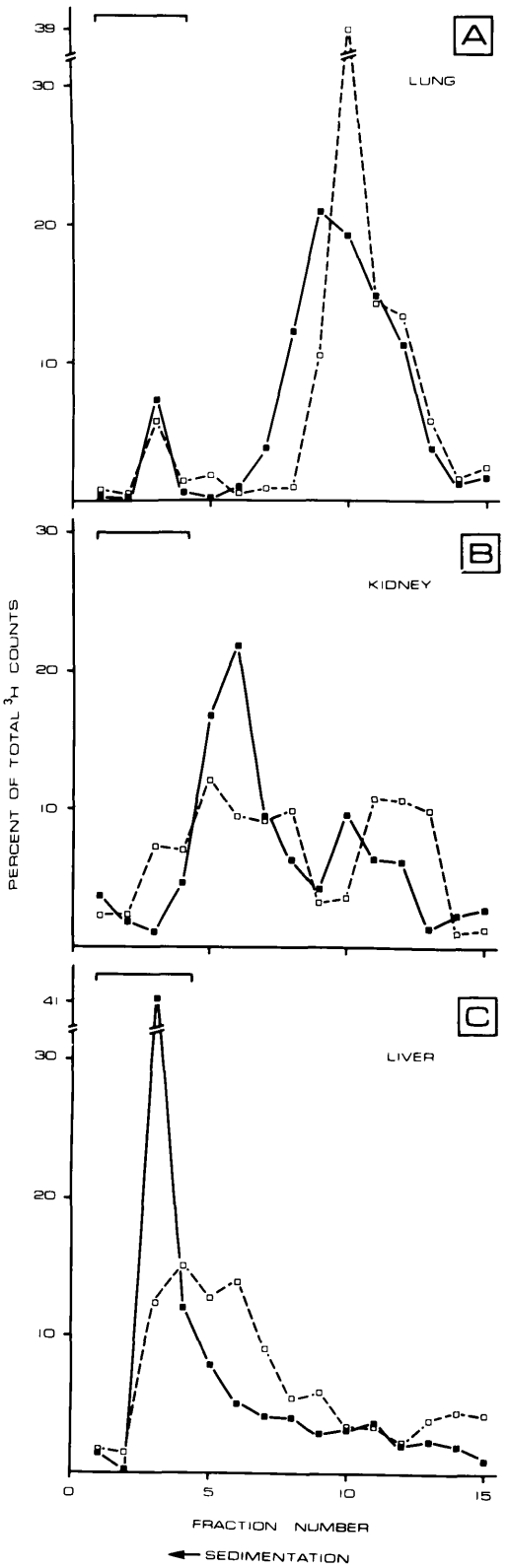
FIG. 1. Alkaline sucrose gradient sedimentation profiles of ^{3}H-DNA released from lung fragments prepared, by the mincing technique, from lung pieces of different wet weights: (A) 0.5–1 mg (●——●), 1–2 mg (○——○) (B) 2–3 mg (■——■), 4–5 mg (□——□). The lung pieces were derived from the same untreated mouse. The horizontal bar indicates the position of control DNA peaks.

7099-S). The minced fragments were drawn into a 20-μl microsampling pipette (Corning) and layered on a gradient.

Alkaline sucrose gradients were prepared 1 hr ahead of time and overlaid with 0.3 ml of lysing solution immediately prior to layering tissue preparations. After layering, and a 30-min lysis period, another aliquot of lysing solution (0.3 ml) was added gently down the walls of the centrifuge tube. The gradients were overlaid with mineral oil and centrifuged. Fractions of 16 drops each were collected and analyzed as previously described (14). The amount of DNA layered per gradient was estimated from aliquots of liver and kidney cell suspensions, and lung tissue fragments, by the standard diphenylamine technique.

Results and Discussion. Lung tissue, because of its tough elastic texture (15) was not amenable to the spatula-squashing method (7), employed successfully in the preparation of liver and kidney cell suspensions. Vigorous mashing of lung tissue with a spatula or tissue-grinder may introduce severe mechanical shearing to constituent DNA and thereby prevent a meaningful comparison from being made with analyses of liver and kidney preparations (Koropatnick, Laishes, and Stich, in preparation). An alternative method of lung tissue preparation was therefore sought.

Sufficient alveolar air was contained in fresh lung tissue fragments to cause the fragments to float on the alkaline lysing solution. A gradual penetration of the lysing solution into small lung pieces (<0.5 mm diam) causes the leached tissue to sink. The sinking tissue fragments were halted at the gradient/overlay interface. Following centrifugation of preparations from control mice, tissue fragments were located in fractions two or three (Fig. 1). Lung tissue fragments were removed from gradients after lysis and also after centrifugation, fixed with (3:1) ethanol:acetic acid and stained with 2% acetoorcein. No intact nuclei were observed. The sedimentation profiles of ^{3}H-DNA released from the lung fragments contained prominent peaks in the control region (7). Reproducible profiles of DNA derived from



the lungs of control mice by the mincing technique were obtained.

A double labeling study was carried out in order to assess the extent of protein contamination of the sedimented ^{3}H-DNA. Sedimentation profiles of DNA from double-labeled mice revealed that 10–20% of the ^{14}C-(protein)-radioactivity cosedimented with lung tissue debris, at fractions 2 or 3. The results indicate that peaks of ^{3}H-DNA are free from gross protein contamination other than that encountered with cosedimenting lung tissue debris.

The results of an experiment designed to compare the extent of DNA fragmentation induced in lung, kidney, and liver of mice injected with 40 mg and 80 mg 4NQO/kg revealed that the extent of DNA fragmentation occurred in the order: lung, kidney, liver, with no detectable liver DNA damage occurring with 40 mg 4NQO/kg (Fig. 2). The relative amounts of DNA being routinely applied to the gradients were estimated (Table I). The results indicated that comparable amounts of DNA, from lung, kidney, and liver, were layered on each gradient. No DNA fragmentation was observed when mice were treated with the nononcogenic 4NQO at doses up to 100 mg/kg.

That DNA fragmentation was actually occurring in the 4NQO-treated cells was suggested by the fact that (a) no shifts in sedimentation profiles were seen when 4NQO (as high as 10^{-3} *M*) was included in the gradient overlay, and (b) no reduction in DNA breakage was observed when 4NQO-treated (80 mg/kg) animals were perfused with EDTA/saline to remove any extracellular 4NQO. In contrast to the action of 4NQO, injections of DMN (1 mg and 8 mg/kg) induced greater DNA fragmentation in mouse liver than in kidney or lung (Fig. 3).

The lung, which showed the greatest 4NQO-induced DNA damage, is also the major site of accumulation and metabolism

FIG. 2. Alkaline sucrose gradient sedimentation profiles of ^{3}H-DNA released from tissues of mice killed 4 hr following 4NQO treatment. 4NQO in DMSO was administered subcutaneously at 40 mg/kg body wt (■——■) and 80 mg/kg body wt (□——□). (A) Lung; (B) Kidney; (C) Liver. The horizontal bar indicates the position of control DNA peaks.

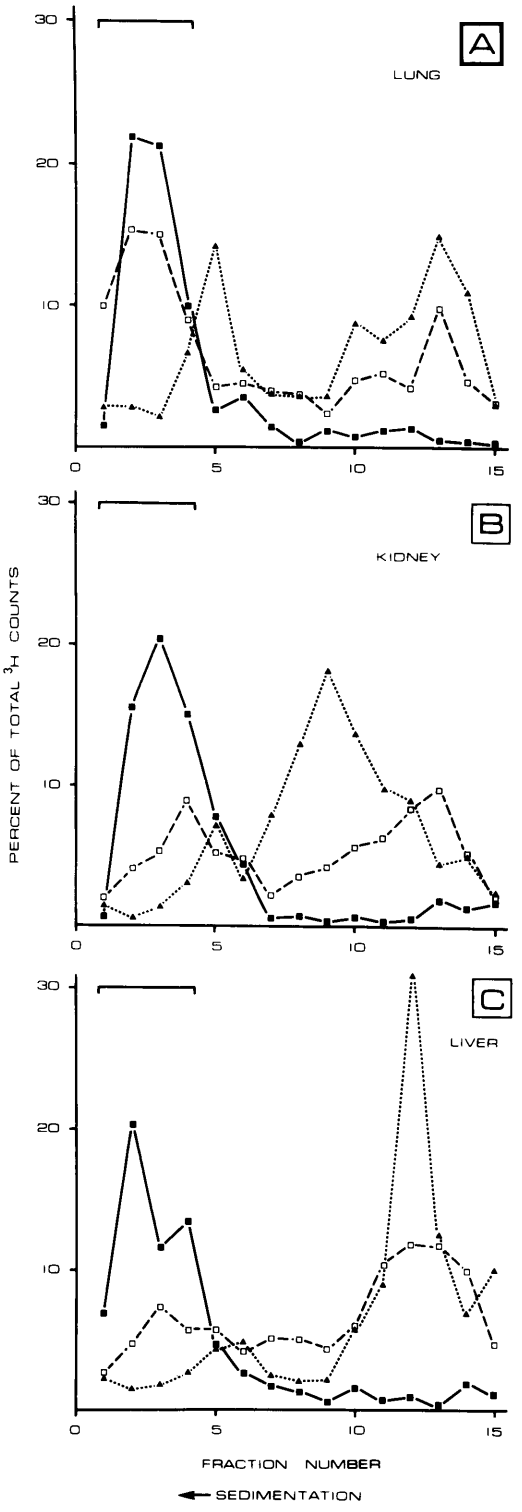


FIG. 3. Alkaline sucrose gradient sedimentation profiles of ^{3}H-DNA released from mouse tissues following no treatment (control) (■——■) and treatment with DMN. DMN, in 0.1 ml 0.9% NaCl, was injected subcutaneously at 1 mg/kg body wt (□——□) and 8 mg/kg body wt (▲——▲). Control animals received 0.1 ml 0.9% NaCl. The mice were killed 4 hr after the administration of DMN or 0.9% NaCl. (A) Lung; (B) Kidney; (C) Liver. Only one set of control profiles is illustrated. The horizontal bar indicates the position of control DNA peaks.

of 4NQO (16), of DNA repair synthesis (17) and tumor formation (4, 16). In this connection it is of interest to note that 4AQO does not induce any detectable DNA fragmentation, nor a DNA repair synthesis, nor neoplasms in lung, kidney, and liver. On the other hand a DNA fragmentation and DNA repair synthesis (17, 18) was observed in all three organs following DMN. The greatest DNA damage was found in the liver, the major site of metabolism of DMN and of tumor formation (5). The results suggest that a DNA fragmentation and DNA repair synthesis occur in the target tissues from which tumors arise following the application of organotropic carcinogens.

Summary. The extent of DNA fragmentation induced in lung, kidney, and liver of mice injected with the chemical carcinogens 4-nitroquinoline 1-oxide (4NQO), dimethylnitrosamine (DMN) and the noncarcinogenic 4-aminoquinoline 1-oxide (4AQO) was estimated by the alkaline sucrose gradient technique. A floating of minced lung tissue pieces in the alkaline lysing solution on top of the gradients afforded a gentle method of lung DNA extraction. This technique minimized mechanical shearing of lung DNA and permitted comparisons to be made with liver and kidney DNA sedimentation patterns. The extent of DNA damage induced by 4NQO followed the order: lung, kidney, liver, while that induced by DMN followed the order: liver, kidney, lung. The sites of greatest DNA damage appeared to correlate with sites of high levels of DNA repair synthesis and the sites of tumor induction. No DNA damage was induced by the noncarcinogenic 4-aminoquinoline 1-oxide (4AQO).

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TABLE I. ESTIMATION OF THE RELATIVE AMOUNTS OF LUNG, KIDNEY, AND LIVER DNA LAYERED PER GRADIENT

Organ	^{3}H-cpm/μg DNA *Run 1*	*Run 2*	*Mean*	Amount of tissue layered/gradient	Range of ^{3}H-cpm in gradients[a]	Amount of DNA/gradient (μg)
Lung	880	850	865	1–2 mg (minced)	800–1000	0.9–1.2
Kidney	800	750	775	1×10^5 cells	600–1100	0.8–1.4
Liver	880			1×10^5 cells	600–1100	0.7–1.3

[a] Range encountered in 40 consecutive experiments.

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