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Use of DNA Repair Synthesis in Detecting Organotropic Actions of Chemical Carcinogens[1] (38009)

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The organotropic effect of chemical carcinogens has been attributed to a metabolic activation of precarcinogens in particular tissues and to an interaction between the ultimate carcinogen and DNA in the target organ (1–3). This action of carcinogens on the genome of differentiated cells is difficult to analyze because of the lack of proper methods. Point mutations of mammalian somatic cells cannot be accurately enumerated. The frequency of numerical and structural chromosome aberrations can be counted, but this approach requires a reasonable mitotic rate, which is frequently lacking in many tissues of adult mammals. In this paper, we report a novel approach to the study of the organotropic action of chemical carcinogens and mutagens. The compounds to be examined are applied *in vivo* and their effect on the DNA of various cell types is estimated by measuring the level of DNA repair synthesis *in vitro*. This idea is based on the assumption that a DNA repair synthesis occurs in those cells in which physical (4, 5) or chemical (6, 7) carcinogens and mutagens have interacted with nuclear DNA, that the DNA alterations are followed by a repair synthesis, and that the level of an unscheduled ^{3}HTdR incorporation is an indicator for DNA repair and thus reveals a preceding DNA damage (8–10).

The two carcinogens which were chosen for this study were 4-nitroquinoline-1-oxide (4NQO) and dimethylnitrosamine (DMN). They differ in their organotropic action. Mice injected with a single dose of 4NQO develop lung adenomas and adenosarcomas at a high frequency (up to 87%), whereas tumors of other organs were only rarely seen with hepatomas absent in all the examined animals (13, 14). In contrast, liver and lung neoplasma and a few kidney tumors develop in mice injected with DMN (15, 16).

Material and Methods. The following schedule was used to estimate a DNA repair synthesis with the proposed *in vivo/in vitro* combination system: 6-week-old male C3/H mice were injected subcutaneously with varying doses of 4NQO (Daiichi Pure Chemical Co., Tokyo), $4NH_2QO$ (courtesy of Dr. Y. Kawazoe), or DMN (Aldrich Chemical Co.) which were dissolved in 9:1 DMSO:EtOH; 2 hr postinjection various organs were removed, teased into tiny pieces, incubated in Eagles Minimum Essential Medium containing 10 μCi/ml tritiated thymidine (^{3}HTdR, New England Nuclear, 20 Ci/mmole) for 3 hr at 37°, fixed with alcohol:acetic acid (3:1), squashed between cover slips and microscopic slide, stained with a 2% acetoorcein solution, repeatedly rinsed in tap water, coated with Kodak NTB-3 emulsion and exposed for 2–3 weeks. For freeze sectioning, tissue pieces were quick frozen after incubation in ^{3}HTdR-containing medium and sectioned on a cryostat. Ten-micron sections were picked up on glass slides and then treated the same as the squash preparations. The incorporation of ^{3}HTdR into nuclear DNA was checked by treating squash preparations with deoxyribonuclease

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prior to autoradiography. Following the enzyme treatment, neither nuclei at S-phase nor nuclei at DNA repair synthesis showed any detectable levels of ^{3}HTdR incorporation. The unscheduled incorporation of ^{3}HTdR into nondividing cells and its autoradiographic detection were employed to reveal the presence of a DNA repair synthesis. This technique has been successfully applied to cultured mammalian cells (4–10), biopsy samples (11), and *in vivo* systems (12). The autoradiographic procedure permits the visualization of DNA repair in individual nuclei, thus facilitating a cytological identification of cell types. Furthermore, the proportion of cells which participate in DNA repair and the variation within a cell population can be readily estimated. Since nuclei engaged in a semiconservative DNA synthesis are heavily labeled or show a clustering of grains over early and late replicating chromosome segments, there is no serious problem of mistaking cells in DNA repair for cells in regular DNA replication (7).

Results and Discussion. The responses of several tissues to the *in vivo* administration of the carcinogens 4NQO and DMN and the nononcogenic metabolite $4NH_2QO$ are summarized in Table I. A DNA repair synthesis does not occur in all tissues but is restricted to cells of particular organs. Also, different cell types of an organ can differ in spite of close proximity, e.g., parenchymal cells and endothelial cells in livers of DMN-injected mice. Within one tissue, not all cells may respond equally. For example, hepatocytes in DNA repair synthesis are not randomly scattered throughout the liver but aggregate in many clusters, following a single application of DMN. Similarly, epithelial cells show an unscheduled uptake in some, but not all bronchi, and DNA repairing cells are found only in a few loops of Henle of mice which received 2 or 3 DMN injections in 30-min intervals. This

TABLE I. DNA Repair Synthesis in Various Tissues Following a Single Injection of the Organotropic Carcinogens 4NQO and the Nononcogenic $4NH_2QO$.

	Average grain number[a]		
Tissue	4NQO 200 mg/kg	DMN 150 mg/kg	$4NH_2QO$
Lung			
epithelial cells	11.5 (78–100%)[b]	6.7 (83–100%)[b]	0
endothelial cells	—	7.2 (88–96%)[b]	—
Liver			
parenchymal cells	0	13.2[c]	0
endothelial cells	0	0	0
Kidney			
glomeruli	0	0	0
loop of Henle	0	4.8[c]	0
Spleen			
Salivary gland	0	0	0
Thymus	0	0	0
Stomach			
epithelial cells	0	0	0

[a] Average grain number of cells which showed unscheduled ^{3}HTdR incorporation; S-phase nuclei were excluded from the counts.

[b] The figures show the variation (%) of nuclei with overlaying silver grains of 10 different mice. It is likely that an unscheduled ^{3}HTdR incorporation occurred in virtually all nuclei. Cells without detectable levels of incorporated ^{3}HTdR could be due to methodological errors: e.g., overlapping of cells on the "squash" preparations or the presence of small nuclei slices on sectioned material.

[c] Cells showing unscheduled ^{3}HTdR incorporation occur in groups.

nonuniform response within a cell population seems to reflect the actual situation rather than being caused by an unequal penetration of ^{3}HTdR into the excised tissue pieces since S-phase cells in semiconservative DNA replication are equally labeled throughout the entire tissue sample.

The patchy distribution of cells in DNA repair makes a quantitative evaluation of the extent of DNA repair in a tissue a difficult task. A dose–response study was performed only on the lung tissue of mice which received two injections of 4NQO (30 min apart). Because up to 90% of all cells showed an unscheduled incorporation of ^{3}HTdR, an accurate grain count on the autoradiographs and estimation of the average grain number per cell was possible. The dose-dependence of DNA repair synthesis is clearly seen in Fig. 1. Furthermore, this study substantiates the organ-specific action of 4NQO. Even at the highest dose of 4NQO, which causes DNA repair in virtually all lung cells, the hepatocytes do not show any detectable levels of unscheduled ^{3}HTdR incorporation.

The results shown in Table I also reveal a different response of liver, lung, and kidney cells towards the potent carcinogens 4NQO and DMN. No detectable levels of unscheduled uptake were seen in tissues of mice injected with $4NH_2QO$, a nononcogenic metabolite of 4NQO.

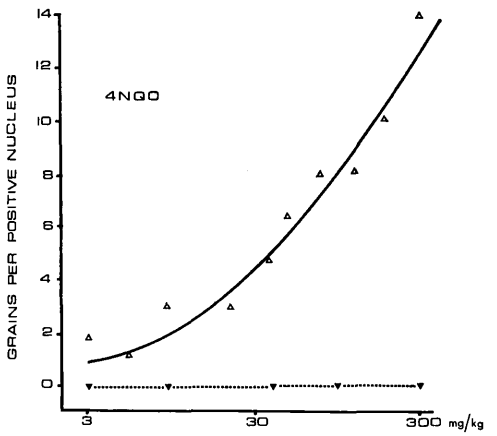

FIG. 1. Unscheduled incorporation of ^{3}HTdR into nuclei of lung cells (△—△) and hepatic parenchymal cells (▼—▼) of mice injected with 4NQO at concentrations varying from 3 to 300 mg/kg.

If the organ-specific DNA repair synthesis following 4NQO and DMN is compared with the metabolism of these carcinogens in various tissues and with the site of tumor formation, a certain correlation seems to emerge. Following injection, 4NQO accumulates in the lung where it is rapidly metabolized into the proximate carcinogen 4HAQO (17), induces a high frequency of lung adenomas and adenocarcinomas (13, 14), and causes a DNA damage in this organ as revealed by a DNA repair synthesis. The liver of these animals contained only traces of 4NQO and 4HAQO (17), no hepatomas were seen in 4NQO-injected animals, and there was no detectable level of DNA repair synthesis in hepatic cells. In contrast, DMN appears to be mainly activated in the liver of rodents, although other organs including kidney and respiratory tracts also appear capable of a low but definite metabolism of nitrosamines (3). DNA repair synthesis occurred in those tissues which give rise to neoplasms: mainly in cells of the liver and lung and in cell clusters of the convoluted kidney tubules.

The simplest interpretation of the results is to assume that an organ-specific metabolic activation of the carcinogens 4NQO and DMN occurred, that these activated carcinogens led to DNA alteration at the site of activation, or at particular sensitive tissues, and that these DNA alterations are, in turn, responsible for the observed difference in DNA repair synthesis among various organs. Whether the correlation between site of carcinogen activation, site of DNA damage, site of DNA repair, and site of neoplasms points to a causal connection is difficult to assess at present. The possibility, however, must be considered that the proposed *in vitro/in vivo* combination test for DNA repair could prove to be a sensitive indicator for organotropic carcinogens and could represent a simple tool for the identification of early carcinogenesis in tissues which may give rise to neoplasms.

Summary. A combined *in vivo* and *in vitro* system for estimating carcinogen-induced DNA alterations was employed to

examine the organotropic action of two carcinogens, 4-nitroquinoline-1-oxide (4NQO) and dimethylnitrosamine (DMN). A DNA repair synthesis, which follows 4NQO- or DMN-induced DNA alterations, was found to occur only in those tissues from which neoplasms arise. No detectable DNA repair synthesis was observed after application of the nononcogenic 4NQO metabolite, 4-aminoquinoline-1-oxide ($4NH_2QO$). This novel *in vivo* and *in vitro* combination system may prove to be a suitable tool to detect organotropic carcinogens and to identify tissues which may give rise to neoplasms.

1. Miller, E. C., and Miller, J. A., Pharm. Rev. **18**, 805 (1966).

2. Lawley, P. D., Prog. Nucleic Acid Res. Mol. Biol. **51**, 89 (1966).

3. Magee, P. N., "Topics in Chemical Carcinogenesis" (W. Nakahara, S. Takayama, T. Sugimure, and S. Odashima, Eds.), p. 259. University Park Press, Baltimore (1973).

4. Rassmussen, R. E., and Painter, R. B., J. Cell Biol. **29**, 11 (1966).

5. Cleaver, J. E., Nature **218**, 652 (1968).

6. Lieberman, M. W., Baney, R. N., Lee, R. E., Sell, S., and Farber, E., Cancer Res. **31**, 1297 (1971).

7. Stich, H. F., and San, R. H. C., Mutation Res. **10**, 389 (1970).

8. Stich, H. F., San, R. H. C., and Kawazoe, Y., Nature **229**, 416 (1970).

9. Stich, H. F., San, R. H. C., Miller, J. A., and Miller, E. C., Nature **238** (1972).

10. Stich, H. F., and San, R. H. C., Proc. Soc. Exp. Med. Biol. **142**, 155 (1973).

11. Lieberman, M. W., and Forbes, P. D., Nature, in press.

12. Epstein, J. H., Fukuyama, K., Reed, W. B., and Epstein, W. L., Science **168**, 1477 (1970).

13. Mori, K., Gann **56**, 513 (1965).

14. Mori, K., and Kondo, M., Gann **57**, 543 (1966).

15. Toth, B., Magee, P. N., and Shubik, P., Cancer Res. **124**, 1712 (1964).

16. Terracini, B., Palestro, G., Gigliardi, M. R., and Montesano, R., Brit. J. Cancer **20**, 871 (1966).

17. Kawazoe, Y., Uehara, N., and Araki, M., Gann **60**, 617 (1969).

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