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Induction of Tumors of the Stomach and Esophagus in Inbred Chinese Hamsters by Oral Diethylnitrosamine[1] (38090)

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With the discovery of the organotropic carcinogenic effects of the nitrosamine type of compounds, it has been possible to induce cancer in animal models at sites mimicking types of cancer seen in man (1–5). The rat strains used most often generally responded to the symmetric dialkylnitrosamines, like dimethyl- or diethylnitrosamine, with cancer in the liver and to a minor extent in other organs such as kidneys and lungs although a few esophageal tumors were sometimes noted. On the other hand, asymmetric dialkylnitrosamines like methylamylnitrosamine typically yield a high incidence of cancer in the esophagus. In the United States, cancer of the esophagus exhibits a low overall rate, but in certain areas black Americans exhibit relatively high rates for unknown reasons (6). Cancer of the esophagus also shows an elevated incidence in some populations in Africa (7, 8), on the east but not the west coast of the Caspian Sea in Iran, and in an area extending from Iran into the Southern Soviet Union and China (9).

Bioassay systems to detect carcinogenic activity usually utilize small rodents such as mice or rats (10). Inasmuch as there is need for improving such animal models, there has been a systematic search for other species with a high sensitivity and a low spontaneous tumor rate. Because virtually all experimental species, including primates, respond well to dimethyl- and diethylnitrosamine, we have customarily utilized these carcinogens to investigate the response of new species (4, 11, 12).

Yerganian (13–15) has developed highly inbred strains of Chinese hamsters. This species is interesting from many points of view, not the least of which is an accurate knowledge of the karyotype, consisting of 22 well-characterized chromosomes. Of interest would be specific changes which might occur in the karyotype from cancers induced in this species.

In this paper we record the fact that diethylnitrosamine administered to inbred Chinese hamsters quite unexpectedly induced mainly tumors in the stomach and the esophagus, and only to a much lower extent in the liver or other sites.

Materials and Methods. Male and female Chinese hamsters, strain M, from the Boston colony (15) were maintained in quarantine for 2 weeks after shipment to Worcester. Groups of 20 male and 20 female animals were then adminstered 40 ppm diethylnitrosamine (DEN) (Eastman Kodak, Rochester, NY) in their drinking water throughout the test. Control animals (5 males, 5 females) received drinking water alone. The animals were housed in litter groups of 2–4 animals for the first 14 weeks and then individually in plastic cages on hardwood shavings with free access to Wayne laboratory meal (4% fat, Allied Mills, Chicago). The animals were exam-

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ined daily, weighed weekly, and killed when survival appeared to be jeopardized. Complete autopsies were performed. Tissues were fixed in 10% buffered formalin solution. All abnormal tissues as well as selected organs such as liver, kidney, and lungs were subjected to histological processing. Sections were stained with hematoxylin and eosin. Representative samplings of liver lesions, principally from biopsies taken on the 160th day of study, were placed in cell culture employing a modified Eagle's MEM (16).

Results. The average survival time of hamsters receiving the carcinogen was approximately 145 days. The first animal died after 105 days. All the surviving animals came to autopsy after 200 days when control animals were killed as well. There was no sex difference in survival time or development of tumors.

Description of lesions. Males and females responded similarly in all aspects, and hence the data are combined. Liver nodules diagnosed as extensive cirrhosis were seen in 60% of the hamsters. On the other hand, hepatocellular carcinoma was present in only 5 animals (2 males and 3 females). A hemangiosarcoma was noted in the spleen of 1 female.

Tumors in the forestomach and esophagus were seen grossly in 100% of the hamsters. Most of them appeared microscopically as papillomas. However, 23% of the gastric tumors were squamous carcinomas and 15% of the esophagus tumors were squamous carcinomas.

Since neither type of tumor was palpable, clinical signs were used to estimate the approximate times of tumor induction. The first signs of possible growths in the esophagus were wheezing and labored breathing, accompanied by coughing. The animals also developed scruffy coats, lost weight, and eventually became moribund and were terminated. First signs of respiratory difficulty occurred in treated females after 7 weeks and in treated males after 11 weeks, which seemed to correspond to a size of the lesion which caused swelling of the esophagus and pressure on the larynx and trachea.

Upon autopsy, the esophagus of treated animals contained white papillary growths which on cross-sectioning were seen to occlude from ½ to ⅞ of the lumen. In the stomach, the fundus (cardiac end) showed multiple white papillary growths, while the remainder of the stomach appeared normal.

Microscopically, the benign growths in the esophagus showed papilliform, hyperplastic epithelium, which was frequently hyperkeratotic. Of the malignant growths, some were *in situ,* while many were characterized by downward growth of squamous cells into the muscularis, pearl formation, and numerous mitotic figures. All changes in the stomach were strictly confined to the mucosa of the forestomach, the remainder of the gastric mucosa being normal. Typically, the tumors were large papillary growths with hyperplastic epithelium exhibiting hyperkeratosis. The squamous carcinomas were papilliferous with invasion of the muscularis and occasional metastases into blood vessels.

Description of cell cultures. Since liver was expected to be the main target of DEN, 5 cell cultures were attempted. Biopsies from abnormal liver fragments gave rise to two rapidly proliferating cell lines. Later histological diagnosis indicated that the lesions taken were cirrhotic. One line was distinctly a diploid fibroblastlike cell culture. The second was solely derived from a nonhepatic, epithelioidlike derivative featuring a pseudodiploid karyotype ($2n = 22$) with trisomy and monosomy for a medium-sized acrocentric and small metacentric chromosome, respectively, in the absence of marker chromosomes. This cell type readily adapted to suspension-type culturing schemes (magnetic spinner and roller). Several morphological features suggested this cell line to be nonneoplastic. In addition, cell culture supernatants, with and without 25 μg/ml of BUDR, were assayed by Dr. E. M. Zimmerman of Microbiological Associates, Inc., for C-type virus specific reverse transcriptase and found to be negative.

Discussion. In most species in which diethylnitrosamine was tested, liver cancer was the salient finding. In addition, this carcinogen, under some conditions, induced extrahepatic tumors, as for example, respira-

tory tract cancers in the Syrian golden hamster (15, 17–19). It is all the more remarkable, therefore, that in the highly inbred Chinese hamster utilized in the current study these organs were not affected appreciably Instead, the stomach and the esophagus developed neoplasms, implying that these organs possessed the biochemical potential to produce the required ultimate carcinogen, according to the nomenclature of Miller (20). Noninbred Chinese hamsters given DEN or dibutylnitrosamine also responded with the production of esophageal and gastric cancers (21, 22).

Thus, this species offers not only an interesting tool to examine the detailed alterations in karyotype during carcinogenesis, but also leads to additional opportunities to study *in vivo* and *in vitro* neoplastic changes in relation to the underlying biochemical and cytological mechanisms.

Summary. Inbred Chinese hamsters receiving 40 ppm diethylnitrosamine in their drinking water over a period of 17–26 weeks developed papillary growths of the esophagus and forestomach in all of the animals. In these lesions, squamous carcinoma was found in 23% of the stomach tumors and in 15% of esophageal growths. Hepatomas occurred in 13% of the animals and hepatic cirrhosis in 60%. Explantation of cirrhotic liver tissue in culture gave an epithelioid cell line with a pseudodiploid karyotype, $2n = 22$. The highly inbred Chinese hamster appears to be a suitable model for studies involving carcinogenesis in the esophagus and stomach.

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