Dibutylnitrosamine Carcinogenesis in Syrian Golden and Chinese Hamsters (35219)

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(Introduced by P. Shubik)

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After application of Diethylnitrosamine (DEN), Syrian Golden hamsters (*Mesocricetus auratus*) develop tumors in the respiratory tract, while the neoplasms occurring in Chinese hamsters (*Cricetulus griseus*) are in the upper digestive system (1). Further comparative investigations in the two hamster species using Dibutylnitrosamine (DBN) were undertaken to clarify problems in lung and urinary bladder carcinogenesis. Tumors of liver, esophagus, and bladder were found in rats after application of DBN. A hypothetical metabolite of DBN, butanol-(4)-butylnitrosamine, induced bladder tumors in these animals as well (2).

Materials and Methods. Three-month-old male hamsters originating from our own breeding colonies were used in this experiment, and received a pelleted diet (Hope Farms, TMB-RMH) and water *ad libitum*. The animals were housed in groups of five in Makrolon cages. LD_{50} values were determined after the method of Weil (3). One hundred Syrian and 100 Chinese hamsters were treated once weekly for life with 300 mg/kg of body weight of DBN (6% in olive oil) administered by stomach tube. The same dose was given subcutaneously to 25 animals of each species. After the animals died, all organs were examined histologically (5% formalin fixation, HE staining).

Results. The LD_{50} of DBN administered subcutaneously to S. G. hamsters was found to be 1750 mg/kg of body weight; in Chinese hamsters the value was 561 mg/kg of body weight. Acute liver dystrophy, as well as circumscribed necrosis in kidneys, adrenals, and heart were the causes of death in this experi-

TABLE I. Alterations in the Urinary Bladder of SG and Chinese Hamsters After Treatment with DBN.

Week of treatment	No. of animals examined				Hyperplasia papillomas[a]				Carcinoma			
	SG hamster		CH hamster		SG hamster		CH hamster		SG hamster		CH hamster	
	ig	sc	ig	sc	ig	sc	ig	sc	ig	sc	ig	sc
15	12	0	10	0	4	0	2	0	0	0	0	0
20	17	1	11	2	5, 1[a]	0	1, 1[a]	0	0	0	0	0
25	7	10	10	7	0	5, 3[a]	2	1	0	1	1	0
30	8	3	22	6	3	1	5, 1[a]	0	0	2	4	1
35	5	5	10	0	2, 1[a]	1	2	0	1	2	4	0
40	5	4	3	0	1, 1[a]	3, 1[a]	0	0	1	0	0	0
45	20	1	0	1	4, 2[a]	0	0	0, 1[a]	0	0	0	1
50	26	1	0	0	13, 6[a]	0	0	0	11	1	0	0
Total no.	100	25	66	16	32, 11[a]	10, 4[a]	12, 2[a]	1, 1[a]	13	6	9	2
%					32, 11[a]	40, 16[a]	18, 12[a]	6, 6[a]	13	24	14	12

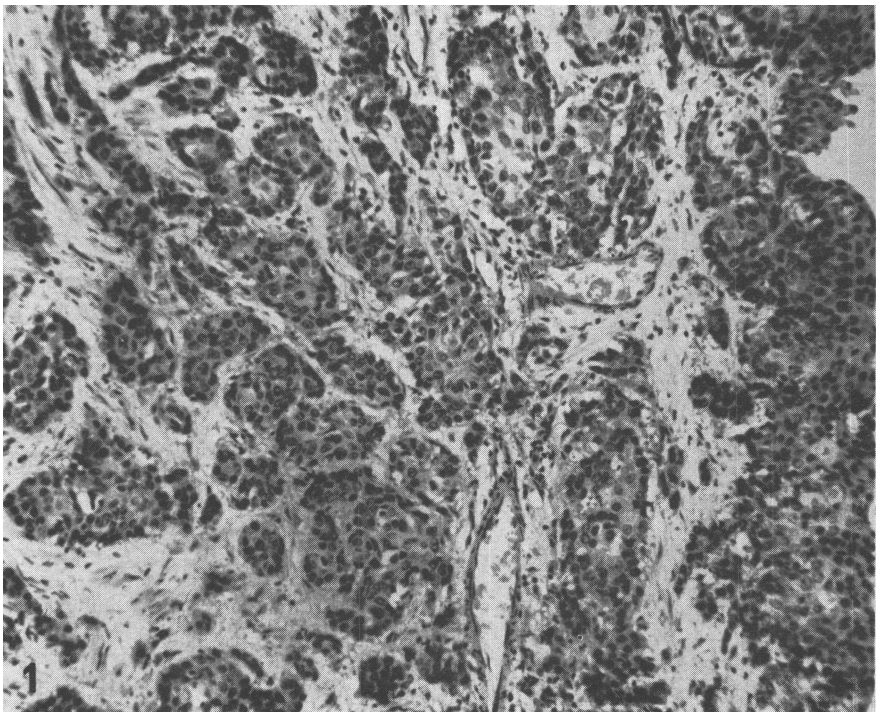

FIG. 1. Transitional cell carcinoma of urinary bladder, Syrian Golden hamster, after DBN treatment (× 115).

ment. Survival times as a function of total dose are shown in Fig. 6. In animals of both species surviving longer than 30 weeks, tumors were found in more than one organ.

Alterations in the urinary bladder. Fifteen weeks after treatment, hyperplasia of the normally one to three layered transitional cell epithelium was seen, especially in the region of the trigone. Afer 23 weeks, papillomas were found. These tumors developed further, and eventually filled the entire lumen of the urinary bladder. In the 33rd week, the first transitional cell carcinoma was observed (Fig. 1).

Histologically, nests of small, densely packed, hyperchromatic cells destroying the basal membranes and infiltrating the subepithelial stroma were seen. In the region of the large tumors infiltrating the muscle layer, bleeding and ulceration were found regularly. Squamous cell carcinoma-like areas were occasionally seen. In addition, in long-surviving animals, ureteric papillomas were found (Fig. 2). Alterations in the urinary bladder are summarized in Table I.

Alterations in the respiratory tract. In the nasal and paranasal cavities of both species, papillomas were seen after 30 weeks, and squamous cell carcinomas were found after 35 weeks. Tumor incidences of 5 to 10%

TABLE II. Tumors of Trachea and Lung in Syrian Golden Hamsters After Treatment with DBN.

Week of treatment	No. of animals examined		Tumors			
			Trachea		Bronchus, lung	
	ig	sc	ig	sc	ig	sc
20	29	1	0	0	0	0
25	7	10	3	9	0	2
30	8	3	5	2	0	1
35	5	5	4	5	1	2
40	5	4	3	4	0	4
45	20	1	3	0	0	0
50	26	1	23	1	13	1
Total no.	100	25	41	21	14	10
%			41	84	14	40

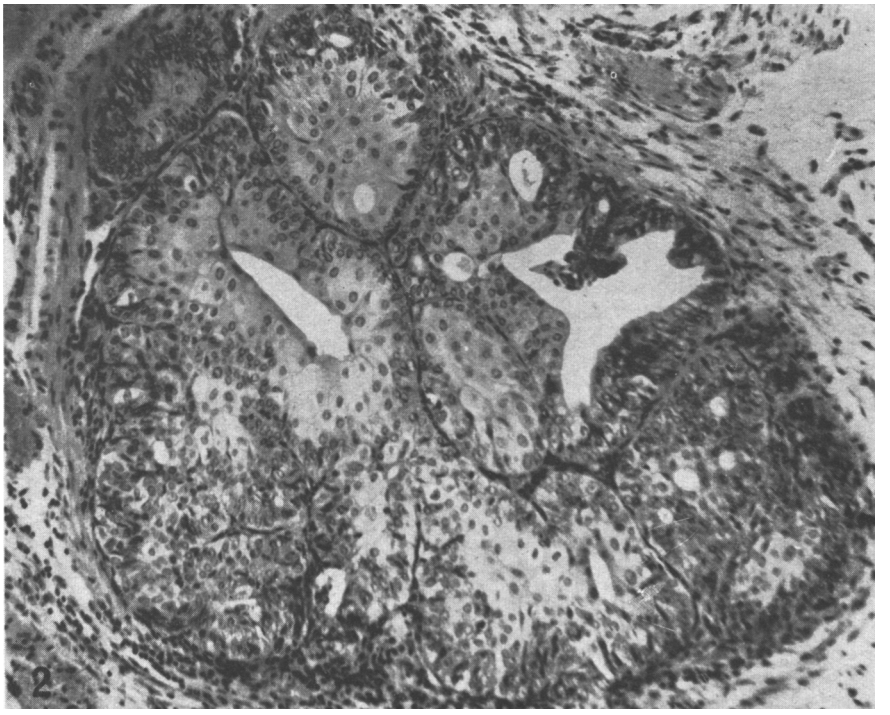

FIG. 2. Papilloma of ureter, Chinese hamster, after DBN treatment (× 115).

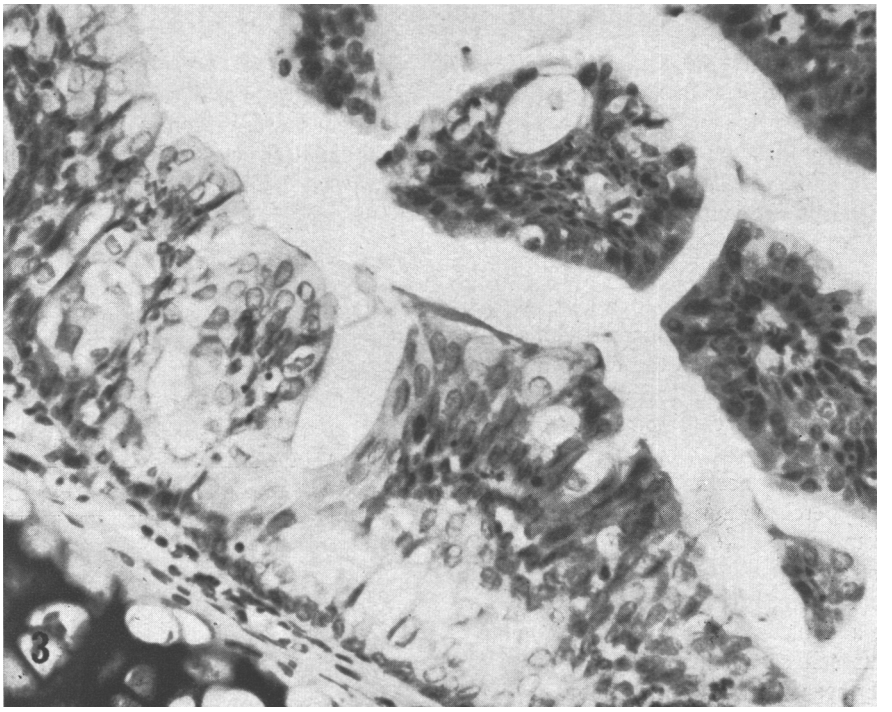

FIG. 3. Hyperplasia and papilloma of trachea, Syrian Golden hamster, after DBN treatment (× 285).

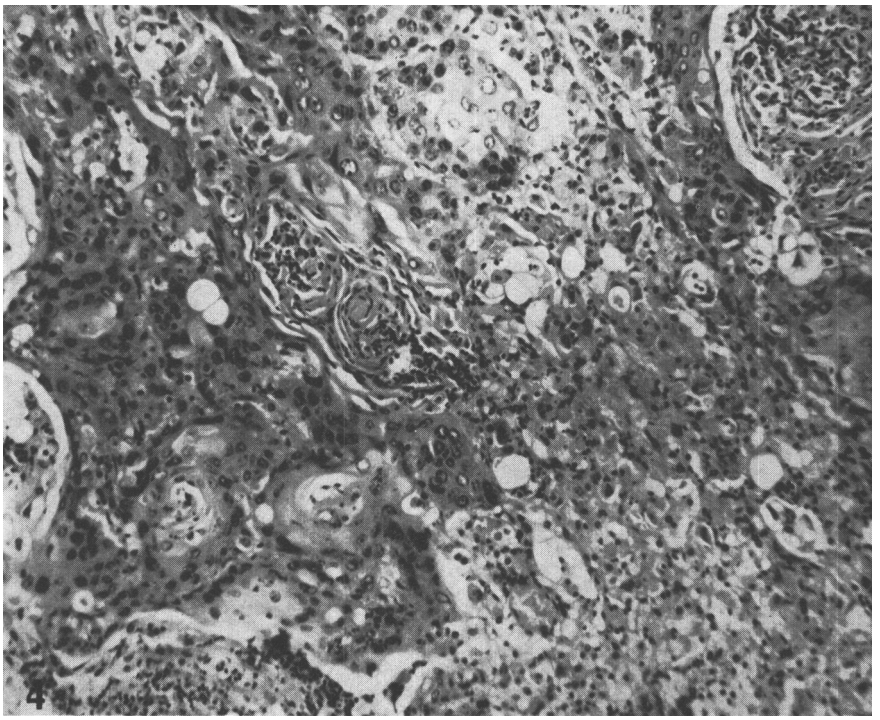

FIG. 4. Keratinizing squamous cell carcinoma of lung, Syrian Golden hamster, after DBN treatment (× 115).

after sc or ig treatment were observed. The first tracheal papillomas in SG hamsters were seen 23 weeks after the beginning of treatment (Fig. 3). Multiple tumors were seen to arise especially in the region just below the larynx and in the bifurcation of the trachea. Three animals showed unkeratinized squamous cell carcinomas of the trachea, while in one animal a squamous cell carcinoma of the plica laryngica was seen (Table II). In Chinese hamsters, however, no tracheal alterations were found.

In the lungs of SG hamsters, treatment resulted in proliferations of the alveolar epithelium (hyperplastic nodules) and occasionally intrabronchial papillary tumors, as well as lung carcinomas 25 weeks after the beginning of treatment (Fig. 4). In the variously differentiated lung tumors, squamous cell formations, with and without keratinization, were occasionally seen. The tumors developed both centrally and peripherally. The classification of these neoplasms as one or another type of carcinoma (squamous cell carcinoma, adenocarcinoma, anaplastic carcinoma) was not unequivocal in any animal. The term "mixed cell type" carcinoma comes closest to the morphological finding. One Chinese hamster showed a bronchial adenoma after 23 weeks while another developed an adenocarcinoma after 36 weeks.

Alterations in the upper digestive tract. Rarely in SG hamsters, but in 25% of the Chinese hamsters, papillomas or squamous cell carcinomas of the oral cavity (mainly in the hard palate) were found after the 30th week of treatment.

Multiple papillomas of the forestomach were commonly seen, as early as the 13th week of treatment, in Chinese hamsters. The same tumors developing in SG hamsters were first seen in the 19th week but were seldom multiple. Histologically, these tumors showed soft, well-vascularized stroma, and well-layered, strongly keratinized squamous cell epithelium. Infiltrating squamous cell carcinomas of the forestomach (Fig. 5) were seen first at the 30th week in Chinese hamsters treated intragastrically (Table III).

Discussion. Small differences in the alkyl

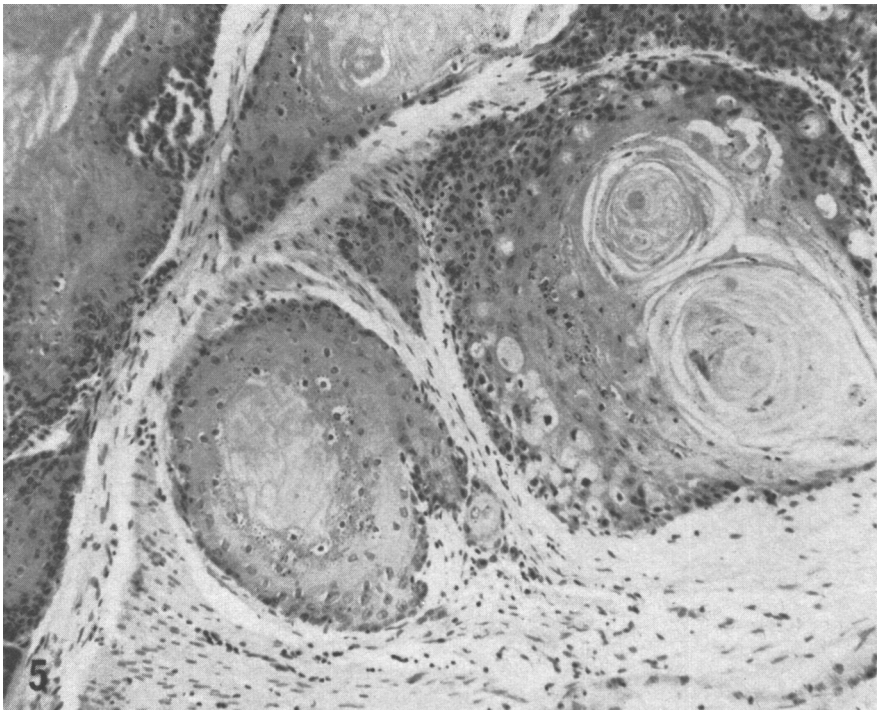

FIG. 5. Keratinizing squamous cell carcinoma of forestomach, Chinese hamster, after DBN treatment (× 715).

groups of nitroso compounds are reputed to result in alterations of their organotropic effect (2). For example, in rats DBN causes tumors mainly in the urinary bladder, a site not favored by other nitroso compounds (4). In the mouse, on the other hand, this substance induces no tumors of the urinary tract (5). In both species of hamsters, treatment with DEN and DBN resulted in similar morphological alterations in the trachea, lung, and forestomach. In this case no different effect was seen with two compounds differing

TABLE III. Alterations in the Forestomach of SG and Chinese Hamsters After Treatment with DBN.

Week of treatment	No. of animals examined				Papillomas				Carcinomas	
	SG hamster		CH hamster		SG hamster		CH hamster		CH hamster	
	ig	sc	ig	sc	ig	sc	ig	sc	ig	sc
15	12	0	10	0	0	0	7	0	0	0
20	17	1	11	2	1	0	10	0	0	0
25	7	10	10	7	0	0	10	3	0	0
30	8	3	22	6	4	1	12	6	8	0
35	5	5	10	0	0	1	0	0	10	0
40	5	4	3	0	1	2	0	0	3	0
45	20	1	0	1	11	0	0	0	0	1
50	26	1	0	0	11	0	0	0	0	0
Total no.	100	25	66	16	28	4	39	9	21	1
%					28	16	59	56	32	6

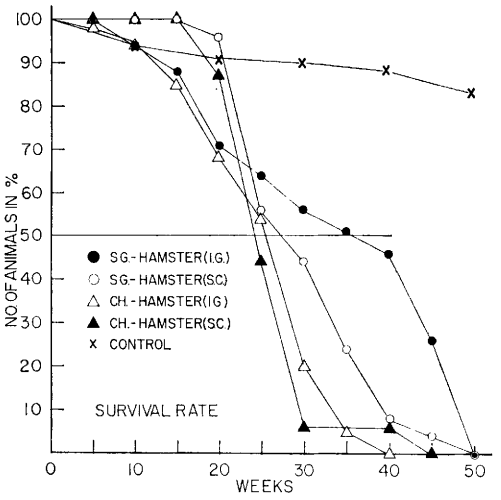

FIG. 6. Survival of SG and Chinese hamsters after ig and sc treatment with DBN.

only in the alkyl group. In addition, both species showed tumors of the urinary tract. It has been suggested that the induction of these tumors is associated with the production of a metabolite (4-hydroxybutylnitrosamine) secreted in the urine and conjugated with glucuronic acid (6).

The morphology of tumors in the respiratory tract, stomach, and bladder does not depend upon the route of application, which influences only the latent periods. Subcutaneous treatment with DBN increases the rate of tracheal and bronchial tumors in SG hamsters, while in Chinese hamsters, the tumor rate in forestomach and bladder is enhanced by intragastric treatment.

Hyperplastic and dysplastic alterations of the epithelium precede the appearance of tumors (8). Malignant tumors appear to arise from papillomas. Therefore, such alterations may be precancers, especially in the nasal cavities, trachea, and bronchi, although most of the animals die by suffocation from benign tumors before the appearance of malignancy. Our studies imply that the mixed cell malignant tumors of the respiratory tract develop from the basal, variously differentiated dysplastic cells.

It was seen that DBN treatment in two hamster species caused progressive alterations of the pseudostatified, ciliated, transitional, and squamous epithelium. This effect could be seen in several different organs at the same time after DBN treatment. Both species showed tumors of the urinary bladder, but the main carcinogenic effect was in the digestive tract in Chinese hamsters and in the respiratory system in Syrian Golden hamsters.

Summary. Comparative studies with Syrian Golden and Chinese hamsters after intragastric and subcutaneous treatment with dibutylnitrosamine are reported. In both species morphologically similar tumors of the urinary bladder, forestomach, oral, and nasal cavities occurred. Syrian Golden hamsters mainly had tumors of the lower respiratory tract, while the Chinese hamsters developed tumors mainly of the digestive tract. The effect in each case was not dependent on the route of administration.

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