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Respiratory Tract Tumours in Hamsters Exposed to Acetaldehyde Vapour Alone or Simultaneously to Benzo(a)pyrene or Diethylnitrosamine*

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Abstract—*Syrian golden hamsters were exposed to* 0 *or* 2500–1650 ppm *acetaldehyde vapour,* 7 hr/day, 5 days/week *for a period of* 52 *weeks. A proportion of the animals was given, simultaneously, either intratracheal instillations of benzo(a) pyrene (BP) or subcutaneous injections of diethylnitrosamine (DENA). All treatments were stopped after* 52 *weeks. The study was terminated after* 81 *weeks. Major effects attributed to acetaldehyde exposure included growth retardation, rhinitis, hyperplasia and metaplasia of the nasal, laryngeal and tracheal epithelium, nasal and laryngeal carcinomas, and a markedly increased incidence of BP-initiated tracheobronchial carcinomas. There was no evidence of acetaldehyde enhancing the development of DENA-initiated tumours of the respiratory tract. It was concluded that acetaldehyde is an irritant, as well as a carcinogen, for the respiratory tract of Syrian golden hamsters. Possible mechanisms of the carcinogenicity of this aldehyde are briefly discussed.*

INTRODUCTION

ACETALDEHYDE has been found in the vapour phase of cigarette smoke in relatively high concentrations [1, 2] and is considered one of the important ciliostatic and cytotoxic components of tobacco smoke [3, 4].

Short- and long-term inhalation studies in hamsters showed that acetaldehyde at levels of 1500 ppm and higher is capable of inducing inflammatory changes and severe hyper- and metaplasia of the epithelium in the upper segments of the respiratory tract [5, 6]. In addition, repeated intratracheal instillations of acetaldehyde in doses up to approximately 60 mg/kg body weight/week resulted in severe epithelial hyperplasia and inflammatory changes in the bronchiolo-alveolar region of the lungs of hamsters [6, 7]. Neoplasms attributable to acetaldehyde were not observed either in the inhalation or in the instillation study. The long-term inhalation study (1500 ppm acetaldehyde, 7 hr/day, 5 days/week during 52 weeks) produced some evidence of acetaldehyde enhancing the development of BP-initiated tracheal tumours [6]. To verify the findings in this experiment, another long-term inhalation study with acetaldehyde was carried out in hamsters, using a higher concentration of the test compound (2500–1650 ppm), two different doses of BP (18.2 and 36.4 mg), given intratracheally, and also diethylnitrosamine (2.1 μl), given by subcutaneous injection. This study is described in the present report.

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MATERIALS AND METHODS

Chemicals and apparatus

Acetaldehyde (obtained from Fluka A. G. Buchs, S. G., Switzerland) was distilled and analysed gas chromatographically to check its purity. The acetaldehyde was evaporated by passing a known nitrogen flow, bubbling from a fritted glass disc, through an evaporation column containing the liquid. The acetalde-

hyde/nitrogen mixture was diluted with an exhauster-generated carrier airflow of conditioned air, which was passed through glass and copper tubing to a 2.5 m^3 cylindrical stainless steel–glass exposure chamber. The chamber has a conical top and bottom and is provided with a hard glass entrance door, a micromanometer to monitor the negative pressure inside and a rotameter-type flow-meter in the exhaust duct. The animal cages are suspended in a heptagonal frame rotatable around the longitudinal axis of the chamber.

The diluted acetaldehyde vapour was passed through the chamber at a rate of 20 m^3/hr. An exposure chamber of the same type, containing the control animals, was supplied with air only. To monitor the acetaldehyde concentration in the test atmosphere, samples were taken automatically at regular intervals by means of a stainless steel sampling loop, using a timer-controlled gas-sampling valve and analysed by gas chromatography.

The benzo(a)pyrene (BP) used was supplied by Fluka A. G., Buchs, S. G., Switzerland; its purity was checked by thin-layer chromatography and appeared to be higher than 99%. It was ground in an agate mortar for about 30 min and then suspended in 0.9% NaCl solution by means of ultrasonic vibration. Two different BP suspensions, containing 0.175 and 0.35% BP (w/v) respectively, were prepared and stored at −20°C in quantities sufficient for one treatment of all animals in a group. Just prior to use, the suspensions were again vibrated ultrasonically for 2–3 min.

Diethylnitrosamine (DENA) was obtained from EGA-Chemie K. G., Keppler and Reif, Steinheim/Albuch, F.R.G. Its purity was checked gas chromatographically. Solutions of 0.0625% DENA in 0.9% NaCl (v/v) were stored at 4°C (at most for a period of 2 months) in quantities sufficient for one treatment of the animals in one group.

0.9% NaCl solution (sterile, non-pyrogenic) was used as supplied by Baxter Laboratories, A. Christiaens N. V., Brussels, Belgium.

Experimental design and conduct

Male and female Syrian golden hamsters, 6 weeks old, were obtained from the randomly bred colony of the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands. They were housed 6 to a cage in tinned wire-screen cages. The temperature in the chambers was 24°C, the relative humidity of the air inside was about 60%. To prevent contamination of the diet with acetaldehyde, the animals were fed a fresh portion of pelleted stock diet (Muracon I, Trouw & Co. N.V., Amsterdam/Putten, The Netherlands) every day after the exposure. They received tap water *ad libitum*, also during the exposures.

Five hundred and four male and 504 female hamsters were evenly distributed, according to body weight, over 4 inhalation chambers: 1 control chamber, in which the animals were exposed to filtered and conditioned air, and 3 test chambers, in 1 of which acetaldehyde vapour was added to the air supplied. The 2 other chambers were used for exposure to furfural and acrolein respectively. The results obtained with these compounds have already been published [8, 9].

Acetaldehyde was dosed at an average concentration of 2500 ppm (4500 mg/m^3 air), 7 hr/day, 5 days/week during the first 9 weeks, 2250 ppm (4050 mg/m^3) during weeks 10–20, 2000 pm (3600 mg/m^3 air) during weeks 21–29, 1800 ppm (3240 mg/m^3 air) during weeks 30–44 and 1650 ppm (2970 mg/m^3 air) during weeks 45–52. The dosage level was reduced several times because of considerable growth retardation and to avoid early mortality of the test animals.

Both the hamsters in the control chamber and those in the test chamber were divided, according to body weight, into 5 groups. Two groups (groups 1 and 2) consisted of 18 males and 18 females each, and each of the other 3 groups (groups 3, 4 and 5) comprised 30 males and 30 females. In addition to exposure to air or acetaldehyde vapour the animals of the different groups were treated as follows: group 1, no treatment; group 2, 52 weekly intratracheal instillations of 0.2 ml 0.9% NaCl solution; group 3, 52 weekly intratracheal instillations of 0.2 ml 0.175% BP in 0.9% NaCl solution; total amount of BP instilled/hamster was 18.2 mg; group 4, 52 weekly intracheal instillations of 0.2 ml 0.35% BP in 0.9% NaCl solution; total amount of BP instilled/hamster was 36.4 mg; group 5, 17 subcutaneous injections of 0.2 ml 0.0625% DENA in 0.9% NaCl solution given every 3 weeks; total volume of DENA injected/hamster was 2.1 μl. BP or DENA were generally administered between 08.00 a.m. and 02.00 p.m.

Before each instillation the animals were lightly anaesthetised with freshly distilled ether. Body weights were recorded every 2 weeks during the first 6 weeks and monthly thereafter. At the end of the exposure period (week 52) 3 males and 3 females, taken randomly out of each of the 2 groups not treated with BP or DENA, viz. groups 1 and 2, were killed by exsanguination from the abdominal aorta after

anaesthesia with a barbiturate given intraperitoneally. Haematological studies were carried out on blood from the abdominal aorta and comprised determinations of haemoglobin concentration, packed cell volume and the numbers of erythrocytes and total and differential leucocytes. In addition, determinations were made of total serum protein and serum albumin, and of the activities of serum glutamic–oxalacetic transaminase, glutamic–pyruvic transaminase and alkaline phosphatase. The weights of the following organs were recorded: heart, kidneys, liver, spleen, brain, gonads, lungs (+trachea and larynx) and adrenals. Tissue samples of these organs and also of the gastrointestinal tract, thyroid, aorta, uterus, pancreas, mesenteric lymph nodes, skin, skeletal muscle, urinary bladder, salivary glands and head (after removal of the skin, brain and lower jaw) were fixed in a 4% neutral formaldehyde solution. The lungs were fixed by intratracheal infusion with the formaldehyde solution under 10 cm water pressure. Following fixation, the heads were decalcified in nitric acid. The tissues and organs mentioned above of all animals were embedded in paraffin wax and sections of 5 μm (4 transverse sections across the nose and 3 longitudinal sections through larynx, trachea with main bronchi and the pulmonary lobes) were stained with haematoxylin and eosin, and examined microscopically.

The experiment was terminated after 81 weeks. Then all survivors were killed and autopsied. Hamsters that died spontaneously or were killed when moribund were also subjected to a thorough autopsy. Autopsy, fixation and histological techniques were effected as mentioned above. Of all animals, the entire respiratory tract, tumours and gross lesions suspected of being tumours were examined histologically.

RESULTS

Body weights and mortality

Hamsters exposed to acetaldehyde had substantially lower body weights than those exposed to air from week 4 and onwards (Table 1). During the post-exposure period (weeks 53–81) the differences in body weight between test and control animals generally diminished, but did not disappear.

Mortality was slightly higher in acetaldehyde-exposed hamsters than in controls (Table 2).

Table 1. *Average body weights of hamsters exposed to air or acetaldehyde vapour and treated intratracheally with BP of subcutaneously with DENA*

Treatments*			Average body weights (g) at the end of week							
Inhalation of:	Intratracheal instillation of:	Subcutaneous injection of:	0	4	14	26	42	52	66	80
		Males								
Air	—	—	85	96	102	106	101	102	98	102
Air	0.9% NaCl	—	84	95	106	112	106	110	113	116
Air	BP (18.2 mg)	—	85	95	102	103	100	103	112	116
Air	BP (36.4 mg)	—	85	96	101	105	105	107	106	113
Air	—	DENA	85	98	107	108	103	104	108	112
Acetaldehyde	—	—	85	87‡	86§	90§	84§	87‡	95	101
Acetaldehyde	0.9% NaCl	—	85	89	86§	87§	83§	87§	95‡	98‡
Acetaldehyde	BP (18.2 mg)	—	84	89§	88§	86§	86§	91‡	99‡	101§
Acetaldehyde	BP (36.4 mg)	—	84	84§	85§	83§	87§	89§	100	98†
Acetaldehyde	—	DENA	84	86§	87§	86§	84§	85§	92§	99‡
		Females								
Air	—	—	86	108	115	119	113	119	115	113
Air	0.9% NaCl	—	86	108	119	116	120	119	120	118
Air	BP (18.2 mg)	—	87	105	115	117	112	106	108	108
Air	BP (36.4 mg)	—	87	101	116	116	110	106	108	110
Air	—	DENA	87	104	115	123	118	117	116	121
Acetaldehyde	—	—	86	97†	94§	96§	91§	94§	105	102†
Acetaldehyde	0.9% NaCl	—	87	96†	98§	101§	97§	97§	94§	94†
Acetaldehyde	BP (18.2 mg)	—	86	94‡	94§	97§	96§	95§	95‡	103
Acetaldehyde	BP (36.4 mg)	—	86	96	96§	100§	95§	99	98	101
Acetaldehyde	—	DENA	86	97	99§	103§	93§	92§	100§	103‡

*At week 52 all treatments were stopped.

†$P < 0.05$; ‡$P < 0.01$; §$P < 0.001$, according to Student's *t*-test. The various groups of acetaldehyde-exposed animals were compared with the corresponding groups of air-exposed controls.

Table 2. Cumulative mortality of hamsters exposed to air or acetaldehyde vapour and treated intratracheally with BP or subcutaneously with DENA

Treatments*				No. of deaths at the end of week						
Inhalation of:	Intratracheal instillation of:	Subcutaneous injection of:	No. of animals per group	4	14	26	42	52	66	80
			Males							
Air	—	—	30†	0	1	1	2	4	5	7
Air	0.9% NaCl	—								
Air	BP (18.2 mg)	—	30	0	0	0	3	6	6	8
Air	BP (36.4 mg)	—	30	0	0	0	3	3	6	11
Air	—	DENA	30	0	0	0	0	0	0	1
Acetaldehyde	—	—	30†	0	0	0	2	6	8	11
Acetaldehyde	0.9% NaCl	—								
Acetaldehyde	BP (18.2 mg)	—	30	0	1	1	3	4	6	11
Acetaldehyde	BP (36.4 mg)	—	30	1	1	1	5	12‡	14‡	20‡
Acetaldehyde	—	DENA	30	0	0	0	0	0	4‡	11§
			Females							
Air	—	—	30†	0	0	0	2	4	5	16
Air	0.9% NaCl	—								
Air	BP (18.2 mg)	—	30	0	1	5	9	13	16	21
Air	BP (36.4 mg)	—	30	0	1	2	7	10	12	18
Air	—	DENA	30	0	1	1	3	3	6	11
Acetaldehyde	—	—	30†	0	1	4‡	7	9	13‡	20
Acetaldehyde	0.9% NaCl	—								
Acetaldehyde	BP (18.2 mg)	—	30	0	0	2	4	5‡	8‡	17
Acetaldehyde	BP (36.4 mg)	—	30	0	0	1	6	13	20‡	23
Acetaldehyde	—	DENA	30	0	0	2	5	8	9	16

*At week 52 all treatments were stopped.
†Initially, both groups together comprised 36 males and 36 females. At week 52, 6 males and 6 females of each group were killed for interim information. These animals are not included in the table.
‡$P < 0.05$; §$P < 0.01$, according to the chi square test. The various groups of acetaldehyde-exposed animals were compared with the corresponding groups of air-exposed controls.

Death rate increased slightly more rapidly in animals treated with BP and exposed to either air or acetaldehyde than in those exposed to air or acetaldehyde alone. In addition, mortality was considerably higher in animals, particularly in males, treated with the high dose of BP and exposed to acetaldehyde than in animals given the same dose of BP but exposed to air. The considerably higher death rate in DENA-treated males exposed to acetaldehyde as compared to the corresponding group of males exposed to air was not ascribed to an effect of acetaldehyde, but to the exceptionally low mortality in the DENA-treated males exposed to air.

Haematology, blood biochemistry and organ weights

There were no significant differences in haematological and biochemical findings between air- and acetaldehyde-exposed hamsters, except for a slight increase in alkaline phosphatase activity in females exposed to acetaldehyde.

The relative weights of the kidneys and lungs were higher in hamsters exposed to acetaldehyde than in controls exposed to air, the differences being statistically significant in females only.

Pathology

Effects of the acetaldehyde exposure alone. In animals killed at the end of the exposure period pathological changes attributable to acetaldehyde were found in the nose, larynx and trachea. The nasal changes consisted in rhinitis, thinning and degeneration of the layer of olfactory epithelium, hyper- and metaplasia of mainly the respiratory epithelium and thickening of the submucosa, which occurred nearly exclusively in the dorso-medial part of the nasal cavity. Metaplastic stratified squamous epithelium, often with heavy keratinisation, was mainly encountered on the naso-maxillary turbinates and on the anterior part of the nasal septum. Actually, the nasal lesions were very similar to, but less severe than, those which have been found previously in hamsters repeatedly exposed to 4560 ppm acetaldehyde

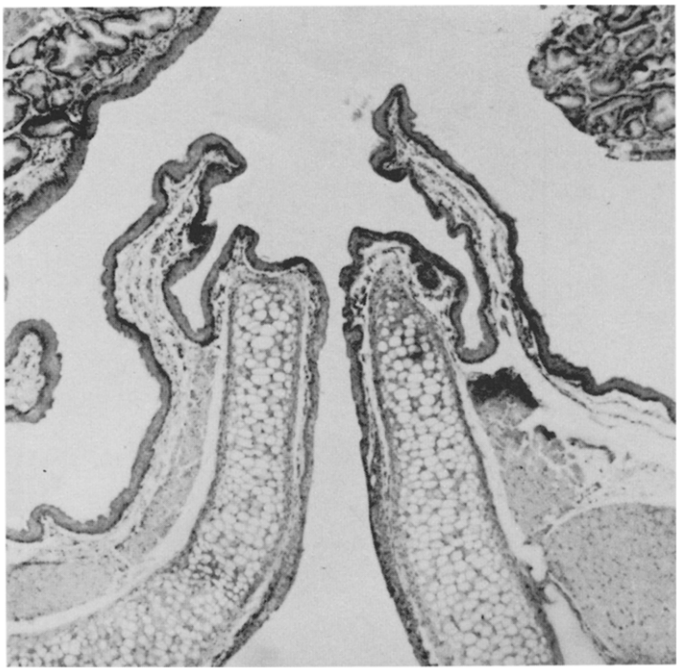

Fig. 1. *Slight hyperplasia and metaplasia of the laryngeal epithelium (male, acetaldehyde alone, week* 53, *H & E,* ×50).

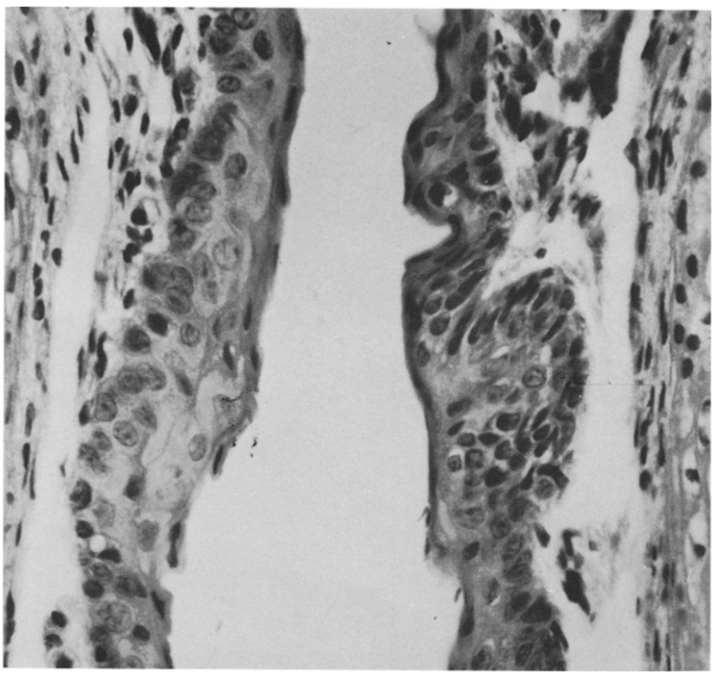

Fig. 2. *Detail of the larynx depicted in Fig.* 1. *Note the irregular pattern of the hyper- and metaplastic epithelium (H & E,* ×430).

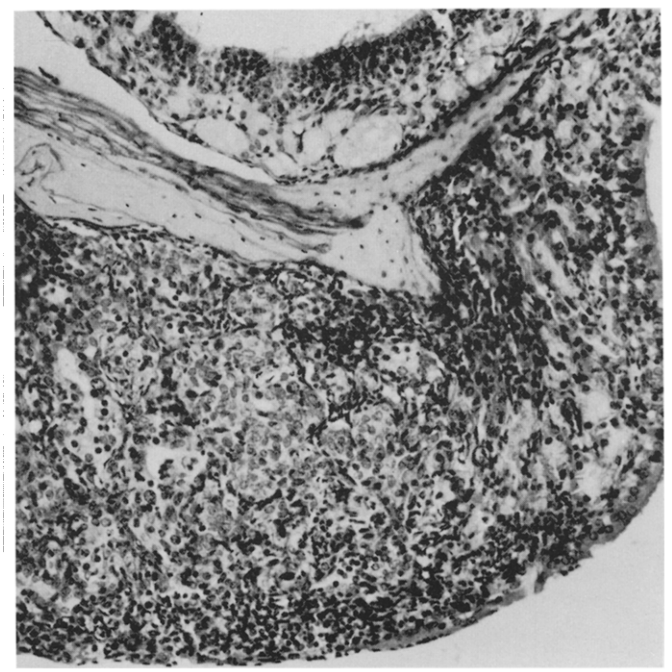

Fig. 3. *Adenocarcinoma of the nose most probably arising from the glands of Bowman (female, acetaldehyde alone, week* 82, *H & E,* ×170).

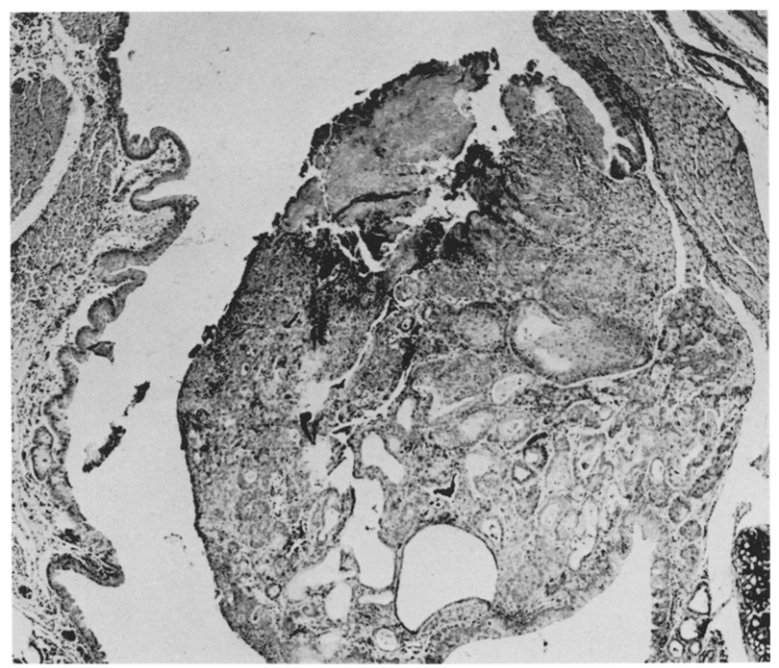

Fig. 4. *Adenosquamous carcinoma of the larynx (female, acetaldehyde alone, week* 78, *H & E,* ×43).

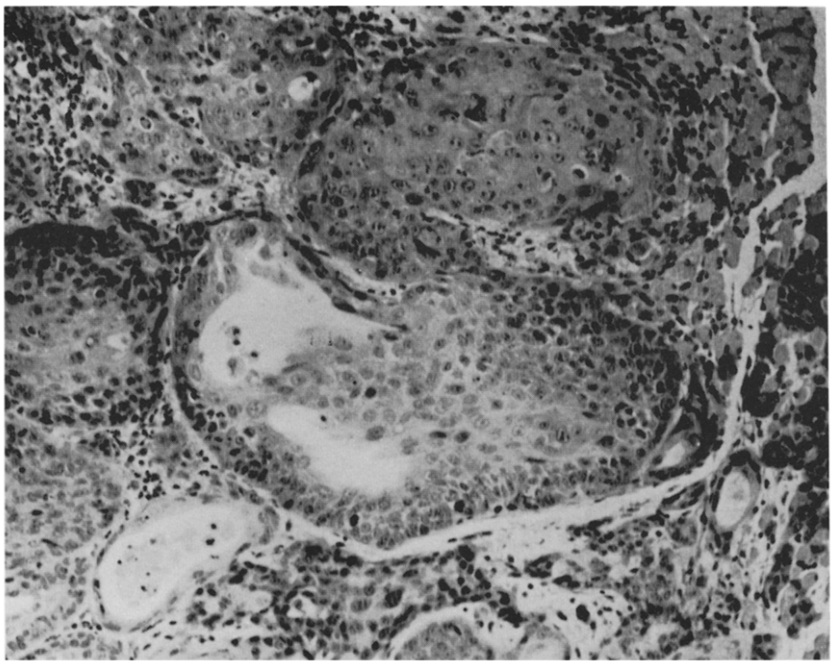

Fig. 5. *Detail of the tumour depicted in Fig.* 4. *Note the adenosquamous pattern and the infiltrative growth* (*H & E*, ×170).

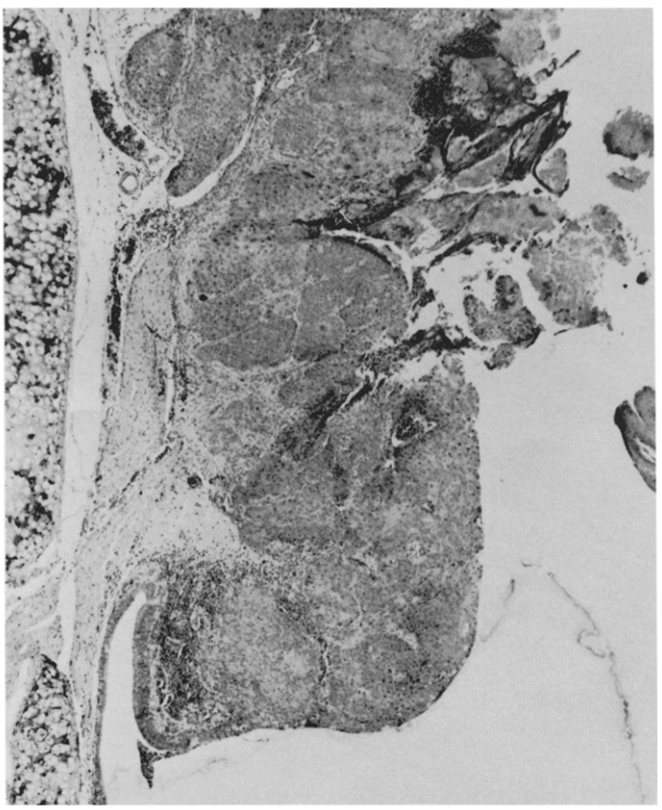

Fig. 6. *Squamous cell carcinoma of the larynx* (*male, acetaldehyde alone, week* 66, *H & E*, ×43).

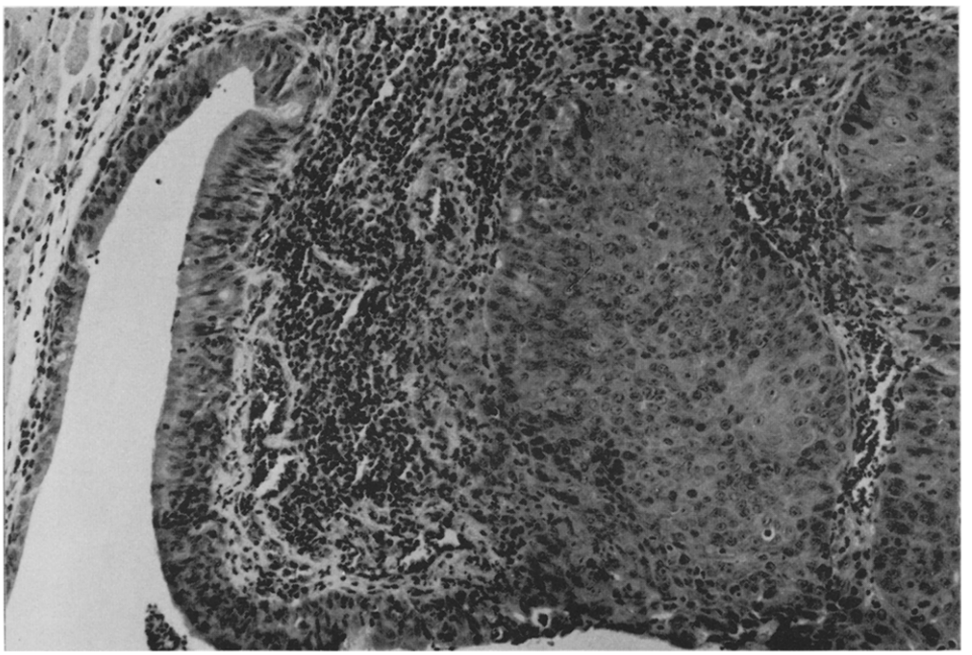

Fig. 7. Detail of carcinoma depicted in Fig. 6. Origin of the tumour from laryngeal epithelium clearly visible (H & E, ×160).

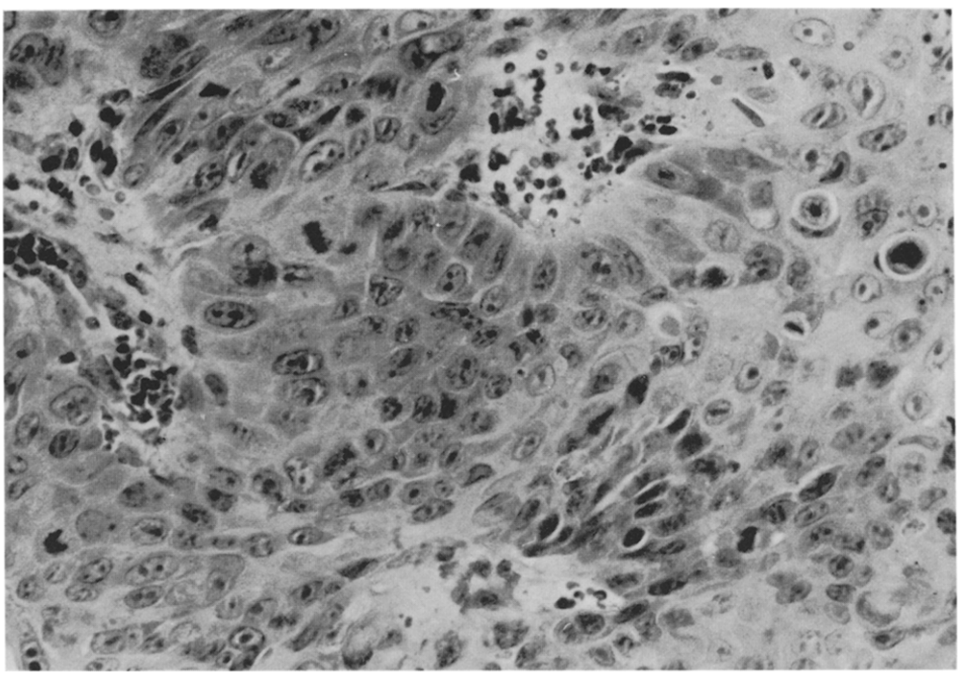

Fig. 8. Detail of carcinoma depicted in Fig. 6. Note cellular and nuclear pleomorphism, prominent nucleoli and mitotic figures (H & E, ×400).

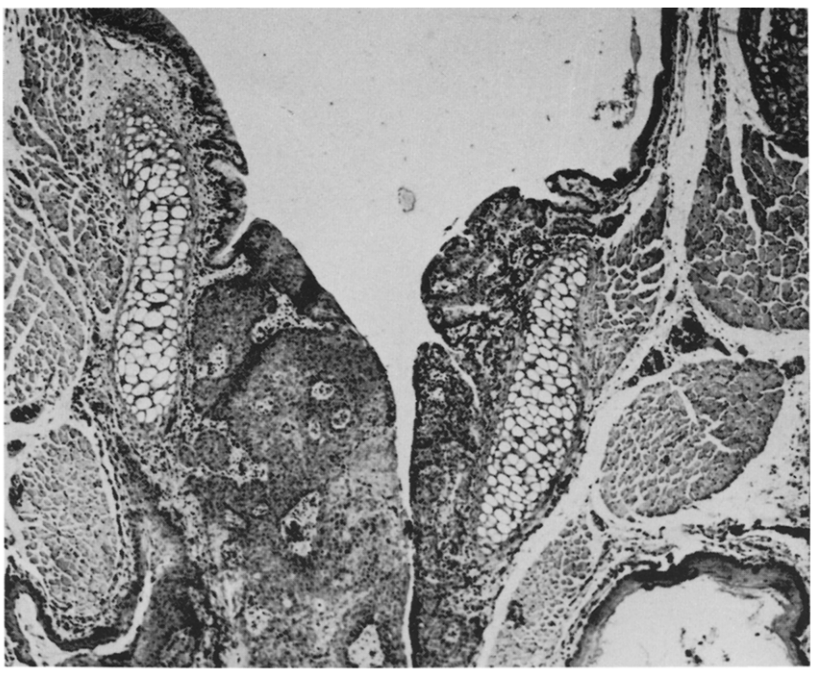

Fig. 9. *Carcinoma* in situ *of the larynx (female, acetaldehyde + DENA, week* 70, *H & E,* ×53).

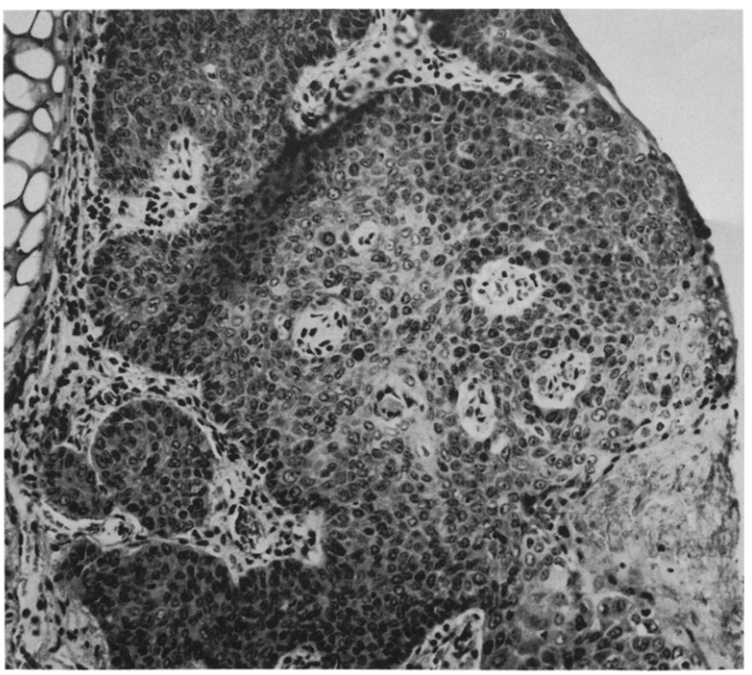

Fig. 10. *Detail of tumour shown in Fig.* 9. *Note the highly atypical structure but absence of infiltration (H & E,* ×210).

for a period of 13 weeks [5]. In both the larynx and trachea slight to moderate focal hyperplasia and squamous metaplasia of the lining epithelium were found in nearly all acetaldehyde-treated hamsters (Figs. 1 and 2).

In one of the females exposed to acetaldehyde the larynx showed a thick layer of irregular, keratinised, stratified squamous epithelium with cellular and nuclear atypia. No tumour was found in any of the hamsters killed at the end of the exposure period.

In animals found dead or killed when moribund, nasal and laryngeal changes occurred (Table 3) which were very similar to those seen in acetaldehyde-exposed animals killed at the end of the exposure period. Many of these animals died or were killed after recovery periods varying from a few weeks to, at most, 6 months. Nevertheless, they showed atrophic, inflammatory, hyperplastic and metaplastic changes in the nose and larynx, indicating the persistence of the alterations involved. In addition to these findings, tumours were encountered in both the nose (adenoma, adenocarcinoma and anaplastic carcinoma) and the larynx (carcinoma *in situ*, squamous cell carcinoma and adeno-squamous carcinoma) of animals exposed to acetaldehyde vapour alone, the incidences being 7 and 26% in males and 4 and 20% in females for the nose and larynx respectively (Tables 3–5; Figs. 3–8). The differences between acetaldehyde-exposed animals and controls exposed to air with respect to the incidence of the various types of these tumours together were statistically significant only in the case of the laryngeal tumours ($P < 0.05$, according to the chi square test). In addition to the neoplasms in the larynx, the laryngeal epithelium of several males (17%) and several females (15%) exposed to acetaldehyde alone showed hyper- and metaplasia with unequivocal nuclear and cellular atypia. The neoplastic and non-neoplastic lesions in the larynx were mainly located either on the vocal cords or in the most anterior part of the larynx; occasionally lesions were found at both sites. In none of the animals exposed to air alone was a nasal or laryngeal tumour or atypical hyper- and metaplasia of the larynx detected (Table 3). The incidence of nasal and laryngeal tumours and of atypical hyper- and metaplasia of the larynx in hamsters exposed to acetaldehyde and treated with either BP or DENA was similar to that found in hamsters exposed to acetaldehyde alone (Tables 3–5). Laryngeal hyper- and metaplasia, carcinoma *in situ* (Figs. 9 and 10) and squamous cell carcinomas were found after combined treatment, but were not observed at all after treatment with either BP or DENA alone. It seems justifiable, therefore, to also ascribe these hyper- and neoplastic lesions, found after the combined treatment, to acetaldehyde alone.

In the trachea minimal focal hyperplasia and metaplasia were seen in only a few hamsters exposed to acetaldehyde alone, indicating the reversible nature of acetaldehyde-induced hyper- and metaplasia of the tracheal epithelium. No tracheal tumours were observed in hamsters exposed to acetaldehyde alone (Tables 4 and 5). In the bronchi and lungs no changes were detected that could be attributed to acetaldehyde exposure alone.

Effects of simultaneous exposure to acetaldehyde and BP. The incidence of carcinomas in the trachea and bronchi was approximately four times higher ($P < 0.001$, according to the chi square test) in hamsters exposed to acetaldehyde and treated with the high dose of BP (36.4 mg) than in hamsters treated with the same dose of BP but exposed to air (Table 5). Moreover, the latent period of tracheo-bronchial carcinomas was much shorter after the combined exposure than after treatment with 36.4 mg BP alone (Fig. 11). Such differences in tumour response of the tracheobronchial epithelium were not found between the group exposed to both acetaldehyde and the low dose of BP (18.2 mg) and the group treated with the low dose of BP alone (Table 5). There was no indication of a stronger tumour response of the nose, larynx or lungs after the combined treatment than after treatment with either acetaldehyde or BP (Tables 4 and 5). Actually, the number of pulmonary tumours in males was much lower ($P < 0.01$, according to the chi square test) after the combined treatment than after treatment with the high dose of BP alone (Table 5). Since BP-induced pulmonary tumours appeared in a late stage of the experiment, this difference might well be due to the relatively high death rate of males exposed to both compounds (Table 2).

Effects of simultaneous exposure to acetaldehyde and DENA. There was no evidence of acetaldehyde exposure increasing the incidence or affecting the type of DENA-induced tumours in any of the segments of the respiratory tract (Tables 3–5). No tracheal tumours were observed in females exposed to acetaldehyde and DENA, whereas 8 out of 27 females treated with DENA alone bore a tracheal tumour. Since the incidence of DENA-induced tracheal tumours may vary considerably, in particular if the dose used is as low as it was in the present study, the absence of tracheal tumours in

*Table 3. Type and incidence of neoplastic and non-neoplastic changes in the nose and larynx of hamsters exposed to air or acetaldehyde vapour and treated intratracheally with BP or subcutaneously with DENA**

Organ and type of changes	Incidence of changes: Inhalation of air				Inhalation of acetaldehyde			
	0.9% –† NaCl‡	BP§ (18.2 mg)	BP‖ (36.4 mg)	DENA¶	0.9% –† NaCl‡	BP§ (18.2 mg)	BP‖ (36.4 mg)	DENA¶
	Males							
Nose	(24)	(28)	(30)	(26)	(27)	(28)	(27)	(29)
Thinning of olfactory epithelium	0	0	0	0	8	7	5	8
Thickened submucosa	0	0	0	0	16	18	18	18
Rhinitis	2	3	2	1	12	9	16	14
Periodontitis	1	0	1	0	0	0	0	0
Ossiferous tissue mass	0	0	0	0	0	0	0	2
Epithelial hyper/metaplasia (a) slight/moderate	0	0	0	2	10	15	12	10
(b) severe	0	0	0	0	4	3	7	5
Epithelial hyper/metaplasia with atypia	0	0	0	0	0	0	0	2
Polyp/papilloma	0	0	0	1	0	0	0	0
Adenoma	0	0	0	0	1	0	0	0
Squamous cell carcinoma	0	0	0	0	0	2	0	3
Adenocarcinoma	0	0	0	1	0	0	1	0
Anaplastic carcinoma	0	0	0	0	1	0	0	0
Larynx	(20)	(28)	(29)	(28)	(23)	(26)	(25)	(30)
Laryngitis	0	0	1	0	2	3	0	5
Epithelial hyper/metaplasia (a) without atypia	1	2	2	5	6	4	3	9
(b) with atypia	0	0	0	0	4	3	7	7
Polyp/papilloma	0	0	1	7	1	1	1	6

Carcinoma *in situ*	0	0	0	0	3	1	2	3
Squamous cell carcinoma	0	0	0	0	2	6	5	1
		Females						
Nose	(23)	(27)	(24)	(27)	(26)	(28)	(22)	(26)
Thinning of olfactory epithelium	0	0	0	0	10	8	8	6
Thickened submucosa	0	0	0	0	18	14	13	12
Rhinitis	0	2	0	2	13	13	11	12
Periodontitis/sinusitis	0	0	0	0	0	1	1	1
Ossiferous tissue mass	0	0	0	0	1	0	0	0
Epithelial hyper/metaplasia (a) slight/moderate	0	0	0	0	10	9	9	9
(b) severe	0	0	0	0	11	11	7	9
Epithelial hyper/metaplasia with atypia	0	0	0	0	0	0	1	5
Polyp/papilloma	0	0	0	0	0	0	0	1
Squamous cell carcinoma	0	0	0	0	0	1	0	1
Adenocarcinoma	0	0	0	0	1	0	0	0
Larynx	(22)	(27)	(24)	(27)	(20)	(23)	(23)	(22)
Laryngitis	0	0	0	1	5	1	1	1
Epithelial hyper/metaplasia: (a) without atypia	0	1	0	0	4	5	8	5
(b) with atypia	0	0	0	0	3	5	7	3
Polyp/papilloma	0	1	0	3	1	2	1	1
Carcinoma *in situ*	0	0	0	0	0	0	2	3
Squamous cell carcinoma	0	0	0	0	1	5	1	3
Adeno-squamous carcinoma	0	0	0	0	2	0	0	0

*The number of animals examined is given in brackets. Animals killed at the end of the treatment-period are not included in this table. For the statistical evaluation (of the tumour data) refer to Table 5.

†No further treatment. ‡Given intratracheally (0.2 ml) weekly during 52 weeks. § Given intratracheally in 52 weekly doses of 0.35 mg. ‖Given intratracheally in 52 weekly doses of 0.70 mg. ¶Given intratracheally in 17 three-weekly doses of 0.125 μl.

Table 4. Incidence and site of respiratory tract tumours in hamsters exposed to air or acetaldehyde vapour and treated intratracheally with BP or subcutaneously with DENA (for type of tumours see Table 5)

Inhalation of:	Treatments: Intratracheal instillation of:	Treatments: Subcutaneous injection of:	No. of animals examined*	No. of animals with respiratory tract tumours (total)	No. of animals with tumours of: nose	larynx	trachea	bronchi	lungs	Total no. of respiratory tract tumours
			Males							
Air	—	—**	15	30¶ 0(0%)	0	0	0	0	0	0
Air	0.9% NaCl†	—	15							
Air	BP (18.2 mg)‡	—	29	4(14%)	0	0	2	1	2	5
Air	BP (36.4 mg)§	—	30	19(63%)	0	1	8	3	13††	27
Air	—	DENA‖	29	12(41%)	2	7	3	3	0	15
Acetaldehyde	—	—	15	29¶ 7‡‡(24%)	2	6‡‡	0	0	0	8
Acetaldehyde	0.9% NaCl†	—	14							
Acetaldehyde	BP (18.2 mg)‡	—	29	12§§(41%)	2	8‡‡	3	1	1	15
Acetaldehyde	BP (36.4 mg)§	—	27	22(81%)	1	9‡‡	14	5	3‡‡	32
Acetaldehyde	—	DENA‖	30	11(37%)	3	10	2	0	0	15
			Females							
Air	—	—	14	28¶ 0 (0%)	0	0	0	0	0	0
Air	0.9% NaCl†	—	14							
Air	BP (18.2 mg)‡	—	27	3 (11%)	0	1	0	1	1	3
Air	BP (36.4 mg)§	—	24	7 (29%)	0	0	3	1	5	9
Air	—	DENA‖	27	11 (41%)	0	3	8	2	0	13
Acetaldehyde	—	—	15	29¶ 5§§(17%)	1	4	0	0	0	5
Acetaldehyde	0.9% NaCl†	—	14							
Acetaldehyde	BP (18.2 mg)‡	—	29	11§§(38%)	1	7§§	4§§	0	1	13
Acetaldehyde	BP (36.4 mg)§	—	29	16(55%)	0	4	10	2	3	19
Acetaldehyde	—	DENA‖	28	8(29%)	2	7	0§§	0	0	9

*A few animals were lost through cannibalism or autolysis.
†0.2 ml weekly during 52 weeks.
‡52 weekly doses of 0.35 mg.
§52 weekly doses of 0.70 mg.
‖Given subcutaneously in 17 three-weekly doses of 0.125 μl each.
¶Animals killed at the end of the treatment-period are not included in this table.
**No further treatment.
††Two animals had more than one type of pulmonary tumour.
‡‡$P < 0.01$; §§$P < 0.05$, according to the chi square test. The various groups of acetaldehyde-exposed animals were compared with the corresponding groups of air-exposed animals.

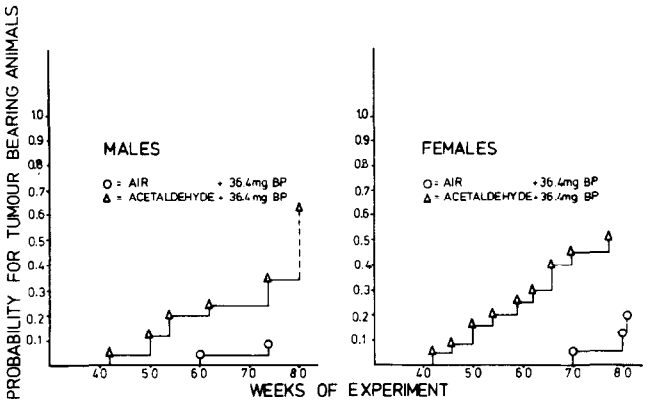

Fig. 11. Probability of observation of a tracheobronchial carcinoma at death of hamsters exposed to air or acetaldehyde and treated intratracheally with 36.4 mg BP (52 weekly doses of 0.7 mg). The probabilities were calculated as described by Saffiotti et al. [10]. *The dashed lines in the last part of two curves indicate that the values of week 81 were not calculated on the basis of the number of animals that died of tracheobronchial carcinoma but on that of the number of animals that were killed and showed this type of tumour.*

females exposed to both compounds is considered a casual finding rather than an indication of an inhibiting effect of acetaldehyde on the formation of DENA-initiated tracheal neoplasms.

Tumours outside the respiratory tract. The following tumours were found in organs other than the respiratory tract: 1 parafollicular cell adenoma, 1 papillary adenoma and 1 follicular adenoma of the thyroid, 1 adrenocortical adenoma and 1 hepatocellular carcinoma. The distribution of these neoplasms over the various groups does not suggest a relationship between the tumours and any of the treatments.

DISCUSSION

The occurrence of inflammatory changes and of hyperplastic and metaplastic epithelial lesions in the upper airways of hamsters following inhalation of acetaldehyde has been previously shown [5, 6] and is fully confirmed by the results of the present study. From one of the previous experiments [6], having a design (1500 ppm acetaldehyde, 7 hr/day, 5 days/week during 52 weeks, followed by an observation period of 26 weeks) very similar to that of the present experiment, it appeared that after a recovery period of 26 weeks the lesions in the upper segments of the respiratory tract had completely disappeared, or were at least markedly reduced in severity and extent. However, the acetaldehyde-induced hyper- and metaplasia of the nasal and laryngeal epithelium found in the study described here, not having changed at all after a 26-week recovery period, indicated their persistent and irreversible nature. Moreover, tumours of the nasal and laryngeal epithelium had developed. Since the main difference between the previous and the present study was a difference in exposure level, it is obvious to assume that the higher level of acetaldehyde used in the present experiment is responsible for the induction of irreversible and neoplastic changes in the nose and larynx. From these findings the conclusion is drawn that acetaldehyde is irritating as well as carcinogenic for the nose and larynx of Syrian golden hamsters.

In the previous long-term study with acetaldehyde [6] the incidence of tracheal carcinomas was higher and their latent period shorter in hamsters exposed to 1500 ppm acetaldehyde and treated intratracheally with 52 mg BP (52 weekly doses of 1 mg each) than in hamsters given the same dose of BP but exposed to air. When lower doses of BP were administered no such enhancing effect of acetaldehyde on the formation of BP-initiated respiratory tract tumours was seen, which led to the conclusion that this aldehyde was not a definite co-factor in respiratory tract carcinogenesis [6]. However, the present observations fully confirm these earlier findings in that evidence was obtained of acetaldehyde strongly enhancing the formation of tracheobronchial carcinomas, again only with the high dose of BP. Therefore, it now seems justifiable to conclude that acetaldehyde is indeed capable of enhancing the development of BP-initiated tracheobronchial tumours in hamsters, provided the dose of BP is sufficiently high.

Recent studies have shown that acetaldehyde may induce crosslinks between DNA strands [11] and also sister-chromatid exchanges in human lymphocytes, as well as in cultured ovary cells of Chinese hamsters [12]. If, indeed, such genetic effects of acetaldehyde were involved in the induction of respiratory tract tumours, as seen in the present study, acetaldehyde should be considered a genotoxic carcinogen. On the other hand, acetaldehyde has

*Table 5. Site, type and incidence of respiratory tract tumours in hamsters exposed to air or acetaldehyde vapour and treated intratracheally with BP or subcutaneously with DENA**

Site and type of tumours	Incidence of tumours							
	Inhalation of air				Inhalation of acetaldehyde			
	−† 0.9% NaCl‡	BP§ (18.2 mg)	BP‖ (36.4 mg)	DENA¶	−† 0.9% NaCl‡	BP§ (18.2 mg)	BP‖ (36.4 mg)	DENA¶
				Males				
Nose	(24)	(28)	(30)	(26)	(27)	(28)	(27)	(29)
Polyp/papilloma	0	0	0	1	0	0	0	0
Adenoma	0	0	0	0	1	0	0	0
Squamous cell carcinoma	0	0	0	0	0	2	0	3
Adenocarcinoma	0	0	0	1	0	0	1	0
Anaplastic carcinoma	0	0	0	0	1	0	0	0
Larynx	(20)	(28)	(29)	(28)	(23)	(26)	(25)	(30)
Polyp/papilloma	0	0	1	7	1	1	1	6
Carcinoma *in situ*	0	0	0	0	3	1	3	3
Squamous cell carcinoma	0	0	0	0	2	6**	5††	1
Trachea	(30)	(29)	(29)	(29)	(28)	(28)	(27)	(30)
Polyp/papilloma	0	2	5	3	0	2	2	2
Squamous cell carcinoma	0	0	1	0	0	1	7††	0
Adenocarcinoma	0	0	0	0	0	0	3	0
Anaplastic carcinoma	0	0	1	0	0	0	0	0
Sarcoma	0	0	1	0	0	0	2	0
Bronchi	(30)	(29)	(30)	(29)	(28)	(29)	(27)	(30)
Polyp/papilloma	0	1	2	3	0	1	0	0
Squamous cell carcinoma	0	0	0	0	0	0	5††	0
Adenocarcinoma	0	0	1	0	0	0	0	0
Lungs	(30)	(29)	(30)	(29)	(28)	(29)	(27)	(30)
Papillary adenoma	0	0	6	0	0	0	0††	0
Acinar adenoma	0	1	3	0	0	0	1	0
Adeno-squamous adenoma	0	1	2	0	0	1	2	0
Adenocarcinoma	0	0	2	0	0	0	0	0

Squamous cell carcinoma	0	0	1	0	0	0	0	0
Anaplastic carcinoma	0	0	1	0	0	0	0	0
			Females					
Nose	(23)	(27)	(24)	(27)	(26)	(28)	(22)	(26)
Polyp/papilloma	0	0	0	0	0	0	0	1
Squamous cell carcinoma	0	0	0	0	0	1	0	1
Adenocarcinoma	0	0	0	0	1	0	0	0
Larynx	(22)	(27)	(24)	(27)	(20)	(23)	(23)	(22)
Polyp/papilloma	0	1	0	3	1	2	1	1
Carcinoma *in situ*	0	0	0	0	0	0	2	3††
Squamous cell carcinoma	0	0	0	0	1	5††	1	3††
Adeno-squamous carcinoma	0	0	0	0	2	0	0	0
Trachea	(28)	(27)	(24)	(27)	(28)	(29)	(28)	(28)
Polyp/papilloma	0	0	1	8	0	3	1	0**
Squamous cell carcinoma	0	0	2	0	0	1	8	0
Anaplastic carcinoma	0	0	0	0	0	0	1	0
Bronchi	(28)	(27)	(24)	(27)	(29)	(29)	(29)	(28)
Papilloma	0	1	0	2	0	0	0	0
Adenocarcinoma	0	0	1	0	0	0	1	0
Adeno-squamous carcinoma	0	0	0	0	0	0	1	0
Lungs	(28)	(27)	(24)	(27)	(29)	(29)	(29)	(28)
Papillary adenoma	0	0	3	0	0	1	2	0
Acinar adenoma	0	0	2	0	0	0	0	0
Adeno-squamous adenoma	0	1	0	0	0	0	1	0

*The number of animals examined is given in brackets. Animals killed at the end of the treatment-period are not included in this table.
†No further treatment.
‡Given intratracheally (0.2 ml), weekly during 52 weeks.
§Given intratracheally in 52 weekly doses of 0.35 mg.
‖Given intratracheally in 52 weekly doses of 0.70 mg.
¶Given subcutaneously in 17 three-weekly doses of 0.125 μl.
**$P < 0.01$; ††$P < 0.05$, *according to the chi square test. The various groups of acetaldehyde-exposed animals were compared with the corresponding groups of air-exposed controls.*

been suggested to be capable of inactivating free cysteine of the bronchial epithelial cells, thereby suppressing the 'thiol-defence' of the epithelium against the attack by mutagens and carcinogens [13, 14]. Such a mechanism might have played a role in the enhancing effect of acetaldehyde on the formation of BP-initiated tracheobronchial tumours by increasing the accessibility of the epithelial cells for BP. Moreover, the strong cytotoxic activity of acetaldehyde is undoubtedly responsible for the hyperplasia, metaplasia and inflammation in the upper airways seen in acetaldehyde-exposed hamsters. Since this process of recurrent tissue damage and repair may possess 'promoting' properties, the carcinogenicity of acetaldehyde may be based on non-specific stimulation of relevant genetic damage caused either by background or endogenous genotoxic carcinogens or initiators. For the time being, it seems justifiable to consider acetaldehyde a carcinogen with a weak initiating and a strong 'promoting' (co-carcinogenic) activity. A more definite classification needs further investigation into the mechanism of action, in particular with regard to the genotoxicity of this aldehyde.

The concentrations of acetaldehyde found in fresh cigarette smoke range from *ca.* 200 to 2000 ppm [2]. It is noteworthy that the exposure level of acetaldehyde in the present study (2500–1650 ppm) was only slightly above the upper limit of this range during the first 6 months of the exposure period and within this range during the last 6 months.

To verify the findings with acetaldehyde in hamsters, and to increase their relevance for humans, it seems indicated to examine the toxicity and carcinogenicity of this compound in another animal species. Therefore, short- and long-term inhalation studies with acetaldehyde in rats have meanwhile been initiated. These experiments seem of particular importance in the light of recent data on the induction of squamous cell carcinomas in the nose of rats by exposure to formaldehyde vapour [15].

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