ponents, for example, collagen, or to nearby cell surfaces and remain intact for an extended period of time; (ii) later, the vesicles undergo endocytosis and are degraded; (iii) if the chelator is NTA, the indium ion is quickly released and binds to a macromolecule, and therefore the radioactivity remains within the cell; or if the chelator is EDTA or DTPA, the complex slowly crosses the cell membrane and the radioactivity is subsequently excreted. Vesicles bearing amino-sugar derivatives of cholesterol could operate by a similar mechanism, but with substantially greater binding constants for polymorphonuclear leukocytes that may account for their transport to the axillary region.

The half-time of vesicle destruction in vivo can be varied over a 50-fold range by the inclusion in the lipid bilayer of various hydrocarbon compounds with charged head groups or sugar and amino-sugar derivatives of cholesterol. This can provide means for controlled deposition and release of therapeutic agents by subcutaneous injection. In combination with the timed release feature, localization of vesicles in tissues may allow visualization or treatment of tumors and metastases of various systems. In addition, observations made with the PAC technique together with studies of the tissue distribution of vesicles containing stable indium complexes may provide considerable insight into the mechanisms of vesicle metabolism. The highly stereospecific effect of the sugar derivatives in targeting these phospholipid vesicles suggests potentially powerful approaches for studying the critical structural features of cell surface receptor molecules.

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Asbestos-Induced Epithelial Changes in Organ Cultures of Hamster Trachea: Inhibition by Retinyl Methyl Ether

Abstract. *The epithelium of the hamster trachea in organ culture undergoes hyperplasia and squamous metaplasia after exposure to the amphibole types of asbestos, crocidolite and amosite. These changes are inhibited when the synthetic vitamin A analog, retinyl methyl ether, is incorporated into the culture medium. These findings suggest a possible use for retinoids in the prevention and treatment of respiratory tract disease associated with environmental exposure to asbestos.*

Occupational exposure to asbestos increases the risk of bronchogenic carcinoma, particularly in long-term cigarette smokers (*1*). The basis for this effect is not known. The amphibole types of asbestos, crocidolite and amosite, induce hyperplastic lesions of basal cells and squamous metaplasia in the differentiated epithelium of organ cultures of hamster trachea (*2*). These cytologic alterations may constitute preneoplastic events.

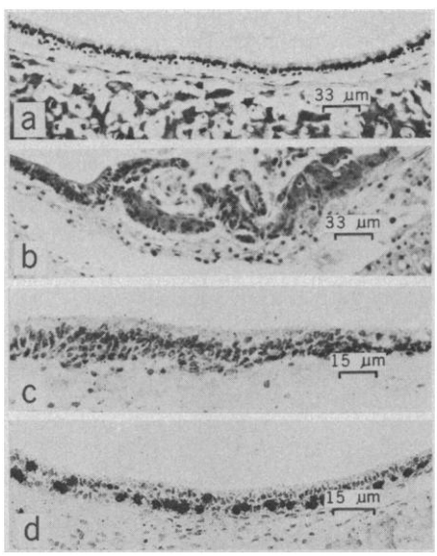

Fig. 1. Epithelium of normal tracheal organ cultures (a and c) and cultures exposed to asbestos (b and d). Note the squamous metaplasia in (b). Autoradiographs of normal and asbestos-treated tracheal epithelia are shown in (c) and (d), respectively. All tissues were maintained in the medium for 3 weeks without retinyl methyl ether.

Synthetic analogs of vitamin A (retinoids) prevent the development of squamous metaplasia and carcinoma in the bladder (*3*), mammary gland (*4*), and respiratory tract (*5*) of animals exposed to chemical carcinogens. Retinoids also reverse the epithelial hyperplasia and squamous metaplasia induced by polycyclic aromatic hydrocarbons in organ cultures of prostatic (*6*) and tracheal tissues (*7*). We now report a similar effect on the changes induced by asbestos in the tracheal epithelium.

Organ cultures are used in our laboratory to evaluate the effects of particulates and fibers, alone and in combination with polycyclic aromatic hydrocarbons, on the differentiated respiratory mucosa (*2*). We then graft tissues into syngeneic hamsters to assess the carcinogenicity of these materials (*8*).

In the experiments reported here, organ cultures were prepared from the tracheas of 60 female golden Syrian hamsters (age, 6 weeks) (15.16 BIO strain, TELACO, Bar Harbor, Maine) (*9*). Tissue from one animal yielded about 15 to 16 individual cultures, each having a mu-

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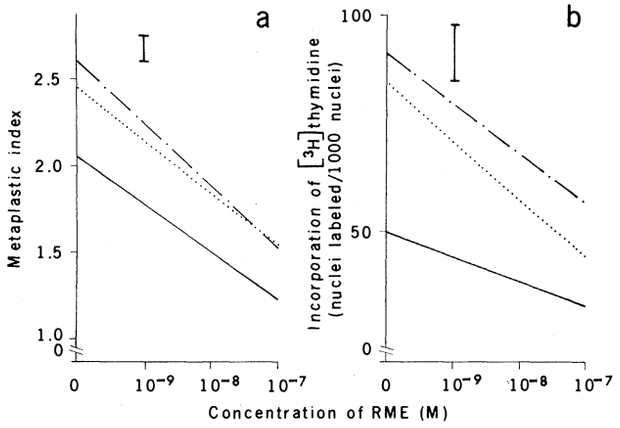

Fig. 2. Retinyl methyl ether (RME) inhibits metaplastic changes (a) ($P < .001$) and the incorporation of [^{3}H]thymidine (b) ($P < .01$) in a dosage-dependent fashion: no asbestos (____, $N = 109$); amosite (—·—, $N = 148$); crocidolite (....., $N = 161$). The results are presented with time as a covariate. The vertical bar represents the estimated standard error (S.E.) of the mean for 35 observations at a given concentration of RME. The statistical method provides an equivalent S.E. of the mean at any point.

cosal surface area of approximately 2 by 4 mm. The explants ($N = 448$) were divided randomly into three groups and placed in 60-mm culture dishes. Suspensions of crocidolite and amosite (International Union Against Cancer reference samples, 4 mg per milliliter of medium) were added to individual dishes. After 60 minutes, the asbestos-containing medium was removed and the tissue transferred to fresh medium (four explants per 35-mm plate). Control explants were submerged in an asbestos-free medium for 60 minutes and treated in a similar manner.

We used the retinoid retinyl methyl ether (RME) for our studies because of its relative lack of cytotoxicity in vitro (*10*). At concentrations of $10^{-6}M$ in culture medium, it induced focal necrosis of the tracheal epithelium. Accordingly, RME was solubilized in dimethyl sulfoxide and diluted in medium to make final concentrations of 10^{-7}, 10^{-8}, and $10^{-9}M$. Controls were maintained in medium without RME. In the amounts used, dimethyl sulfoxide had no effect on the morphology of or the [^{3}H]thymidine uptake by the tracheal epithelium.

Organ cultures were maintained at 35°C in a humid environment consisting of 95 percent air and 5 percent CO_2; we used Eagle's minimum essential medium containing 100 μg per milliliter of gentamicin sulfate and 25 units per milliliter of nystatin. Under these conditions the tracheal mucosa maintains a columnar, ciliated epithelium for 4 weeks or more, although focal proliferation of normal basal cells occasionally is noted (*9*). After 2, 3, and 4 weeks, individual explants ($N = 16$ to 18 per group) were pulsed with [^{3}H]thymidine for 5 hours and fixed in buffered formalin. Alternate histological sections 5 μm thick then were prepared for morphological assessment and autoradiography.

Two dependent features, the development of squamous metaplasia and cellular incorporation of [^{3}H]thymidine, were evaluated. One of us (B.T.M.) assessed the metaplastic changes in the epithelium, using a four-point ordinal scale (*3*). To determine the numbers of epithelial cells incorporating [^{3}H]thymidine, 1000 epithelial cell nuclei (basal and suprabasal) were counted per specimen (*9*). Histological imperfections and sporadic microbial contamination of cultures (apparently unrelated to treatment) precluded examination of identical numbers of tissues in various experimental groups.

Foci of hyperplasia consistently developed in the epithelia of cultures exposed to crocidolite and amosite. As might be expected, this proliferative process was associated with an increase in the uptake of [^{3}H]thymidine by basal cells (Fig. 1). It was prominent after the explants had been maintained in culture for 2 and 3 weeks, at which time squamous metaplasia of the superficial layers of epithelial cells was evident.

We evaluated the data by using an analysis of covariance (*11*). Significant increases ($P < .05$) in both metaplasia and the incorporation of [^{3}H]thymidine were observed (Table 1). Labeling with [^{3}H]thymidine decreased in the tracheal epithelium of all experimental groups with time in culture ($P < .001$) (*9*). Metaplastic changes ($P < .001$) and uptake of [^{3}H]thymidine ($P < .01$) were inhibited with increasing doses of RME (Fig. 2). Multiple comparisons between individual experimental groups demonstrated the maximal effectiveness of RME at $10^{-7}M$ (*12*).

The mechanism whereby amphibole asbestos induces epithelial hyperplasia and squamous metaplasia is obscure (*2*). However, similar cellular events are believed to enhance carcinogenesis since cells undergoing DNA synthesis are transformed more frequently by chemical carcinogens than nonproliferating cells (*13*). The incidence of bronchogenic carcinoma is substantially greater in asbestos workers who smoke than in the normal population (*1*). Thus, the induction of proliferation and metaplasia in the

Table 1. Increases in metaplasia and in the incorporation of [^{3}H]thymidine (mean ± standard error) in tracheal organ cultures exposed to asbestos. At weekly intervals, histological sections 5 μm thick were prepared; these sections were evaluated by autoradiography and the metaplasia was assessed. We determined the degree of incorporation of [^{3}H]thymidine by counting 100 epithelial cells on each side of the center of the explant in each of five serial sections (a total of 1000 cells per tissue). For evaluation of the metaplastic changes in the epithelium, specimens were scored. Explants exhibiting a ciliated columnar epithelium and no metaplasia were classified as 1. Metaplastic changes affecting less than about 15 percent of the epithelium were considered focal metaplasia (= 2). Specimens with greater than 15 percent but less than 50 percent metaplasia were designated as moderately metaplastic (= 3), whereas those lesions affecting more than 50 percent of the epithelium were defined as extensive (= 4). We evaluated the data by covariance analysis (*11*), using the concentration of RME as a covariate.

At the end of week	Number of tissues	Metaplastic index (mean ± S.E.)	Incorporation of [^{3}H]thymidine (mean ± S.E.)
		No asbestos	
2	54	1.48 (± 0.098)	75 (± 12.67)
3	55	1.76 (± 0.110)	22 (± 10.20)
4	30	1.63 (± 0.139)	25 (± 10.10)
		*Amosite**	
2	61	1.70 (± 0.092)	104 (± 11.93)
3	48	2.38 (± 0.118)	75 (± 10.92)
4	39	2.13 (± 0.122)	31 (± 8.82)
		*Crocidolite**	
2	54	1.82 (± 0.098)	88 (± 12.68)
3	53	2.31 (± 0.112)	70 (± 10.39)
4	54	1.92 (± 0.103)	24 (± 7.50)

*Group values for both metaplasia and the incorporation of [^{3}H]thymidine were different from the no asbestos group at $P < .05$ simultaneous level of significance.

respiratory mucosa by asbestos could account for its cocarcinogenic effects in combination with the polycyclic aromatic hydrocarbons in cigarette smoke.

It is unclear how RME prevents hyperplasia and metaplasia, although the retinoid appears to inhibit either cellular division or the synthesis of DNA (or both). In rats, retinoids inhibit the development of squamous lung nodules and carcinomas induced by 3-methylcholanthrene (*5*, *14*) and squamous metaplastic lesions and carcinomas appearing in the urinary bladder after administration of *N*-methyl-*N*-nitrosourea (*3*). These observations and our findings suggest a possible role for retinoids in either the prophylaxis or treatment of respiratory lesions associated with asbestos exposure in man.

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Retina-Dependent Activation by Apomorphine of Metabolic Activity in the Superficial Layer of the Superior Colliculus

Abstract. *Studies of the effects of the dopamine agonist apomorphine on local cerebral glucose utilization by means of the carbon-14–labeled deoxyglucose method demonstrate a dose-dependent metabolic activation in the superficial layer of the superior colliculus in the rat. Apomorphine stimulated glucose utilization in a number of other cerebral structures, but only the effect in the superficial layer of the superior colliculus depended on an intact retinal input. This effect was present with the animal in the light or in the dark, but was abolished by enucleation, which left the effects in other cerebral structures unimpaired. Activation of the superficial layer of the superior colliculus appears, therefore, to be secondary to an action of apomorphine on dopaminergic systems within the retina.*

The 2-deoxyglucose (2DG) technique for mapping functionally related alterations in glucose utilization in discrete regions of the central nervous system (CNS) is a useful tool for studying the central components of the visual system (*1*). Application of the 2DG technique in primates has provided striking demonstrations of the metabolic organization of the primary visual system (*1*), for example, the ocular dominance and orientation columns described originally on the basis of anatomical and electrophysiological studies by Hubel and Wiesel (*2*). In the rat it has provided evidence of a direct quantitative relationship between the level of sensory input from the retina and the rate of glucose utilization in components of the central visual system (the dorsal lateral geniculate body, the stratum griseum superficiale of the superior colliculus, and the primary visual cortex) (*3*).

The existence of dopaminergic neurons among the amacrine cells of the retina has been demonstrated and characterized by fluorescence histochemistry and morphological techniques (*4*). Although the anatomy (*4*) and pharmacological responsiveness in vitro (*5*) of this system are typical of model dopaminergic systems, the physiological significance of the retinal dopaminergic neurons to the visual process remains obscure. In the course of investigations with the 2DG technique (*6*) on the local metabolic consequences of pharmacological manipulation of cerebral dopaminergic systems, we have obtained results which may provide insight into the role of the retinal dopaminergic system in the processing of visual information.

In the first series of experiments in rats under ambient laboratory lighting conditions (approximately 600 lumen/m^2 at eye level), we examined the alterations of local cerebral glucose utilization associated with apomorphine administration. Apomorphine produced significant dose-dependent alterations in the rate of glucose utilization in a number of regions of the CNS. The rate of local glucose utilization was approximately proportional to the logarithm of the dose of apomorphine in the range of 0.15 to 1.5 mg per kilogram of body weight. Glucose utilization increased in the extrapyramidal system (zona compacta and zona reticulata of the substantia nigra, caudate nucleus, globus pallidus, and subthalamic nucleus) and decreased in the anterior cingulate cortex and the lateral habenular nucleus. Qualitative evidence of stimulation of glucose utilization by apomorphine in structures of the extrapyramidal system has previously been reported by Brown and Wolfson (*7*). In our quantitative studies, however, effects were also observed in central components of the visual system. The administration of apomorphine resulted in marked elevations of metabolic activity within the superior colliculus; no statistically significant effects were observed in other components of the CNS with primary visual functions, that is, the dorsal lateral geniculate body and visual cortex (Table 1).

Table 1. Mean rates (± standard error) of glucose utilization (in micromoles per 100 g per minute) in central components of the intact visual systems of normal animals studied in ambient light. Measurement of glucose utilization was initiated 10 minutes after intravenous administration of apomorphine (1.5 mg/kg).

Structure	Saline (N = 6)	Apomorphine (N = 4)
Visual cortex	92 ± 4	90 ± 2
Dorsal lateral geniculate body	68 ± 3	66 ± 3
Superior colliculus	67 ± 4	96 ± 1*

*Significantly different from saline control groups, t-test for group comparison, $P < .01$.

The rate of local glucose utilization in the primary visual structures is directly related to functional activity within the visual system. Visual stimulation is associated with increased glucose utilization in the visual cortex, superior colliculus, and lateral geniculate body; removal of

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