PRETUMOROUS LESIONS AND LUNG AND PLEURAL TUMOURS INDUCED BY ASBESTOS IN RATS, SYRIAN GOLDEN HAMSTERS AND *MACACA MULATTA* (RHESUS) MONKEYS

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Of various carcinogenic pollutants of the human environment, asbestos has been paid particular attention of late, and the problem of 'Asbestos-Cancer' has acquired greater importance than ever. Humans come into contact with asbestos both professionally and in daily life, and its fibres are found in urban air, soil, water, food, etc. (Cunningham & Pontefract, 1971; Nicholson & Pundsack, 1973; Sébastien et al., 1976).

It is beyond doubt that in persons with occupational exposure to asbestos, there is an increased incidence of lung cancer and of pleural and abdominal mesotheliomas. There is some evidence that the incidences of other cancers are also increased, although this requires further study.

There has been no agreement so far on the extent of the carcinogenic hazard to the general population of air, water, soil and food polluted by asbestos. However, it may be assumed that asbestos fibres contribute, with other carcinogenic substances (products of smoking, etc.), to the increased incidence of lung cancer. A certain increase in the incidence of pleural mesothelioma may be expected as well.

Experimental data (Wagner, 1970) demonstrate different activities of asbestos of different types and of the same type when mined in different places. This points to the importance of studying the carcinogenicity of many samples of asbestos and of asbestos dust.

PLAINTIFF'S EXHIBIT

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The main method used to study the carcinogenicity of Soviet asbestos was intrapleural injection in rats. In our opinion, this is an adequate route because in humans asbestos exposure is characterized by pleural mesotheliomas; the increased incidence of this disease is taken by some authors (Selikoff & Hammond, 1978) as an index of environmental pollution with asbestos fibres.

We studied milled commercial asbestos, dust from filters and synthetic asbestos; the dispersion of the samples studied was approximately similar (over 90% of particles were up to 5 μm in length). Asbestos was injected into the right pleural cavity of rats (20 mg thrice monthly in 0.5 ml saline). The results are summarized in Table 1.

Table 1. Carcinogenic activity of asbestos samples after intrapleural injections in rats

Type of asbestos	Basic chemical composition	Character of sample		Pleural mesotheliomas ( )
Chrysotile	$Mg_3(Si_4O_{10})(OH)_8$	Natural	Commercial	65.5 ± 5.5
			Dust from electric filters	46.3 ± 6.1
		Synthetic		2.4 ± 2.4
Amphiboles	$Na_2MgFe_5(Si_4O_{11})_2(OH)_2$	Natural magnesia-arphvedsonite	Commercial	77.1 ± 5.4
	$Mg_7(Si_4O_{11})_2(OH)_2$	Natural anthophyllite	Commercial	41.4 ± 9.1
			Dust from sleeve filters	64.9 ± 7.8
	$Na_2Mg_6(Si_4O_{11})_2(OH)_2$	Synthetic Na, Mg-hydroxy-amphibole		54.5 ± 7.5

Pleural mesotheliomas were induced by commercial chrysotile in 65.3% of animals; their incidence was considerably higher than that induced by chrysotile dust from electrofilters (46.3%). The experiment with synthetic chrysotile resulted in weak carcinogenic activity (2.4% had pleural mesotheliomas). Commercial amphibole asbestos - magnesia-arphvedsonite (MAPh) - induced pleural mesotheliomas in 77.1% of rats. The differences in tumour incidence between commercial chrysotile and MAPh are insignificant. Commercial anthophyllite appeared to be remarkably less carcinogenic (41.4% of pleural mesotheliomas); however, the anthophyllite dust from the sleeve filters was more carcinogenic (64.9%) than milled commercial asbestos.

Synthetic hydroxyamphibole induced pleural tumours in 54.5% of rats, i.e., in considerably fewer than MAPh. Pleural tumours were seen in all experiments with natural asbestos and with synthetic hydroxy-amphibole, from eight months after exposure; the maximal number occurred in the animals that died 400-600 days after the start of the experiment.

The results of our studies on natural asbestos are comparable with those obtained by other authors, both in the percentage of tumours developed and in the relative carcinogenicity of asbestos samples. Commercial chrysotile and MAPh have similar carcinogenicity, which is much greater than that of commercial anthophyllite. These differences in carcinogenic activity of asbestos dust are probably due to the admixture of rock present in the samples.

We also tested asbestos suspensions by intratracheal instillation in Syrian golden hamsters, since this is closer to the natural way in which asbestos is absorbed in humans.

Milled commercial and synthetic chrysotile were injected in doses of 10 mg in 0.2 ml saline twice monthly. Milled commercial MAPh and synthetic sodium-magnesium-hydroxyamphibole were instilled at a dose of 5 mg in 0.2 ml saline twice monthly. The results are presented in Table 2. In comparison with its synthetic analogue, natural chrysotile induced many more respiratory-tract tumours (23% *versus* 63%), and, in particular, malignant tumours (7.7% *versus* 44.4%), although the percentage of benign lung tumours was the same. One pleural mesothelioma was diagnosed in the experiment with natural chrysotile.

The rate of lung tumours induced by natural amphibole asbestos was somewhat higher than that of its synthetic analogue (80.8% *versus* 63.4%), but the differences were not statistically significant. These experiments also resulted in a higher rate of malignant lung tumours after MAPh injections (46.2% *versus* 19.2%). Although amphibole asbestos was given at half the dose of chrysotile, the rate of lung tumours was higher; this confirms the higher carcinogenic activity of amphibole asbestos.

Thus, natural asbestos appears to be more carcinogenic than its synthetic analogue and this is particularly evident with pleural injections in rats. No reason for this difference has been seen so far; it may be due to metal admixtures in the natural asbestos, although this requires further investigation.

Injections of benzo[*a*]pyrene plus asbestos into rat lung stimulated a higher rate of lung tumours than did separate injections of the substances alone (Pylev, 1972). No similar effect was found after injection of asbestos (20 mg) alone or in combination with adsorbed benzo[*a*]pyrene (0.4 mg) into the pleural cavity of rats: the percentage of pleural mesotheliomas was similar in the two experiments (48.6% and 41.4%).

Table 2. Carcinogenic activity of asbestos samples after intratracheal injection into Syrian golden hamsters

Type of asbestos	Character of sample	No. of animals		Animals with tumours (lung, pleura)										Other tumours
		At start of experiment	Effective no. of animals	Total		Benign		Malignant						
						Lung		Lung		Pleura		Total		
				No.		No.		No.		No.		No.		No.
Chrysotile (10 mg twice)	Natural commercial	44	27	17	63.0 ±9.3	5	18.5	11	41.0	1		12	44.4	4
	Synthetic	32	13	3	23.0 ±11.7	2	15.3	1	7.7	-	-	1	7.7	1
Amphibole (5 mg twice)	Natural commercial magnesia-arphvedsonite	30	26	21	80.8 ±7.7	9	34.6	12	46.2	-	-	12	46.2	1
	Synthetic Na, Mg-hydroxyamphibole	56	52	33	63.4 ±6.7	23	44.2	10	19.2	-	-	10	19.2	1

On the basis of these experiments and of other data, we can conclude that there may be two different processes for the carcinogenic action of asbestos, i.e., direct - by its own carcinogenicity, and indirect - by adsorbtion of carcinogenic substances from different sources.

Apart from tumours, asbestos induced various other lesions in lung tissue and pleural mesothelium, which we regard as pretumorous. Similar lesions were found in *Macaca mulatta* (rhesus) monkeys after instillations of chrysotile dust into the lungs and pleural cavity. Up to the present time, seven experimental animals and three control monkeys have died (Table 3). Lesions similar to human asbestosis were also found in experimental monkeys, which can be characterized as diffuse and focal sclerosis in lung tissue and pleura.

Pretumorous lesions and lung tumours induced by asbestos were similar in rats and hamsters. There were focal nodular proliferations of bronchial and alveolar epithelium; and in rats these proliferations were usually solid, with squamous-cell metaplasia. No squamous-cell metaplasia was observed in focal proliferations in hamsters (Fig. 1).

Benign lung tumours were represented by adenomas and papillomas (epithelioma). In rats, 'inversive' lung papillomas with remarkable keratinization were found frequently. Malignant tumours were also of two kinds: adenocarcinoma (Fig. 2) and squamous-cell (epidermoid) carcinoma (Fig. 3).

Pleural mesothelial cells can transform themselves from epithelium-like cells to mesenchymal and fibroblast-like cells. Diffuse hyperplasia and proliferation of pleural mesothelium are the earliest pre-mesotheliomatous lesions. They appear unevenly and occupy large areas of the parietal and visceral pleura. In some cases, the pleural surface

Table 3. Lung and pleural lesions induced by intrapleural and intratracheal injections of chrysotile into *Macaca mulatta* (rhesus) monkeys

Treatment	Quantity of asbestos (mg)	Survival time (months)	Cause of death at autopsy	Morphological lesions
Intrapleural injections of asbestos	107.5	4.5	Pneumonia	Pleural sclerosis, asbestos granulomas, diffuse and focal hyperplasia of mesothelial cells
	581	21.5	Pneumonia, pleuritis	" " "
	987	38	Pneumonia, pleuritis	" " "
	836	38.5	Pneumonia, pleuritis	" " "
Intratracheal injections of asbestos	421	17	Pneumonia	Asbestos granulomas, focal sclerosis, focal hyperplasia of bronchial epithelium
	379	17	Not found	" " "
	609	22.5	Intestinal obstruction	" " " Diffuse hyperplasia of mesothelial cells
Controls - intratracheal injections of saline	-	14.5	Pneumonia, pleuritis	Exudative pneumonia
	-	30.5	Pneumonia	Interstitial pneumonia
	-	31.5	Not found	" " "

FIG. 1. FOCAL HYPERPLASIA AND PROLIFERATION OF BRONCHIAL EPITHELIUM AFTER INTRATRACHEAL INJECTION OF ASBESTOS INTO A SYRIAN GOLDEN HAMSTER (× 150)

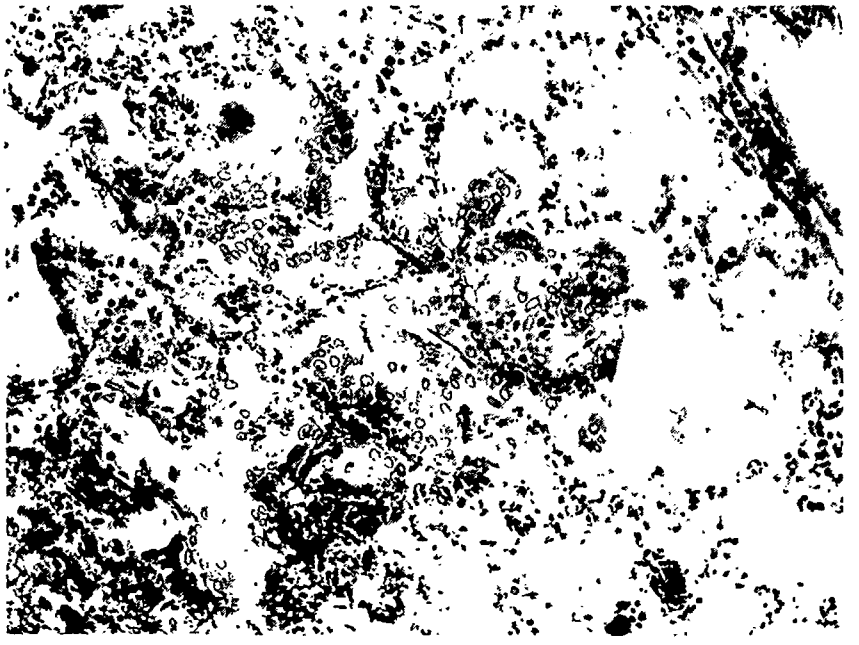


FIG. 2. LUNG ADENOCARCINOMA INDUCED BY ASBESTOS IN A SYRIAN GOLDEN HAMSTER (× 150)

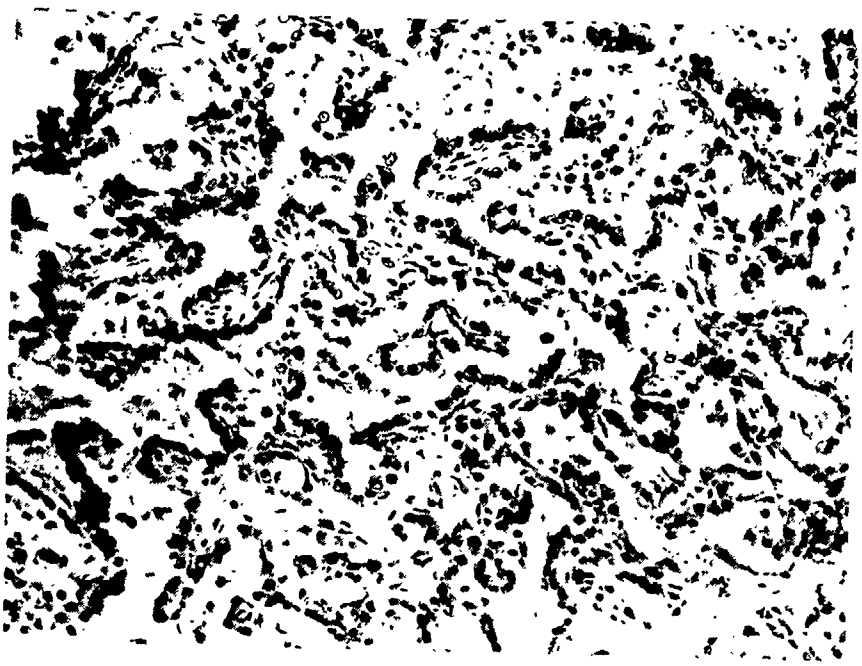
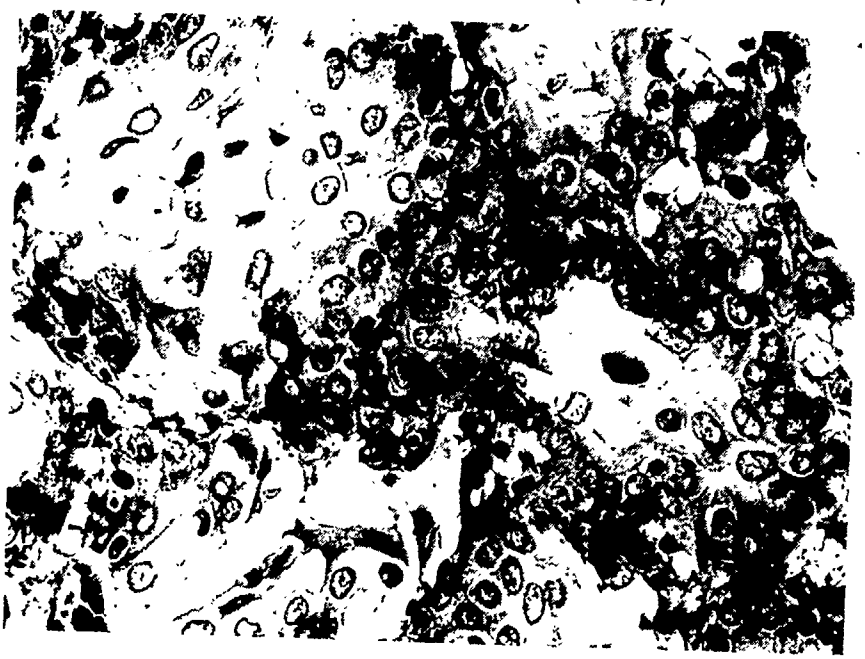
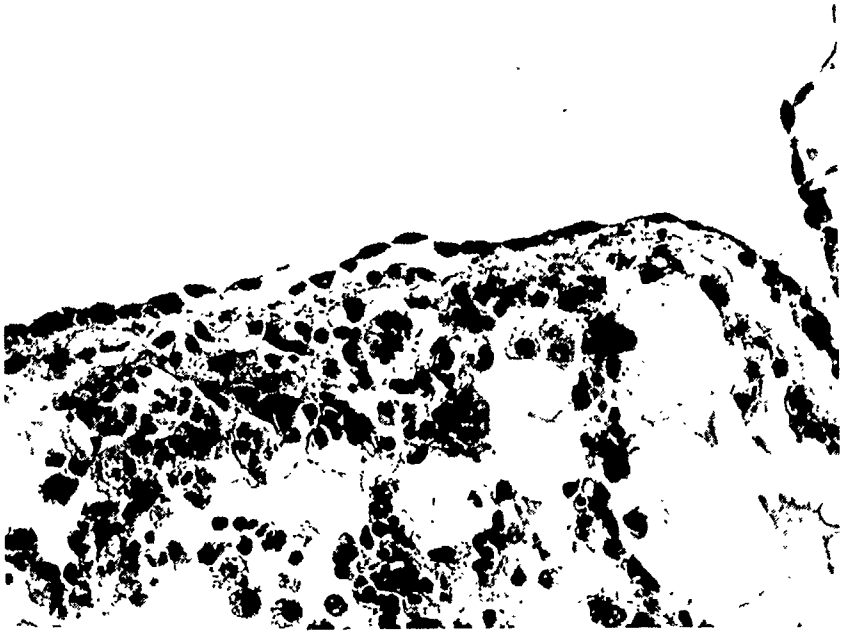


FIG. 3. EPIDERMOID LUNG CANCER INDUCED BY ASBESTOS IN A SYRIAN GOLDEN HAMSTER (× 300)



is covered by cuboidal cells of different sizes and forms, with basophilic cytoplasm and either light or hyperchromatic nuclei. Such lesions were classified as hyperplasia of epithelium-like cells of the mesothelium. We sometimes observed transformation of a number of epithelium-like cells into fibroblast-like cells. In other cases, we found cells that looked like fibroblasts and assumed them to represent diffuse hyperplasia of fibroblast-like cells of the mesothelium (Fig. 4).

FIG. 4. DIFFUSE IRREGULAR HYPERPLASIA OF FIBROBLAST-LIKE PLEURAL MESOTHELIAL CELLS INDUCED BY ASBESTOS IN A SYRIAN GOLDEN HAMSTER (× 150)



Another pretumorous lesions induced in the pleural mesothelium by asbestos injections is focal cell proliferation, which is usually multicentric and distinguished by considerable polymorphism. As with diffuse lesions, these foci are developed by epithelium-like and fibroblast-like cells of the mesothelium. Fibroblast-like cells mostly developed solid structures, and epithelium-like cells developed solid (Fig. 5), adenomatous and papillary lesions. We frequently observed mixed structures of epithelium-like and fibroblast-like cells with adenomatous and papillary growth. Focal proliferations were also observed within asbestos granulomas (Fig. 6).

Hyaluronic acid was found in cytoplasm and in intercellular vacuoles.

FIG. 5. FOCAL HYPERPLASIA AND PROLIFERATION OF EPITHELIUM-LIKE PLEURAL MESOTHELIAL CELLS INDUCED BY ASBESTOS IN A SYRIAN GOLDEN HAMSTER (× 150)

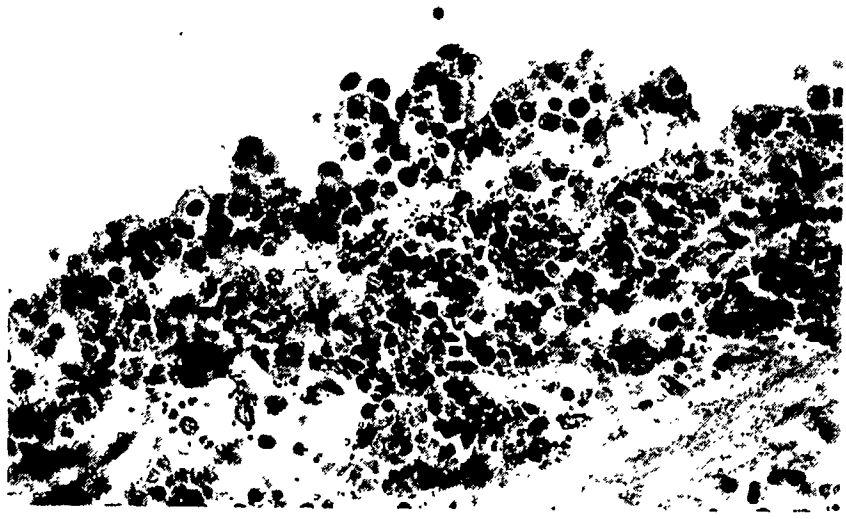
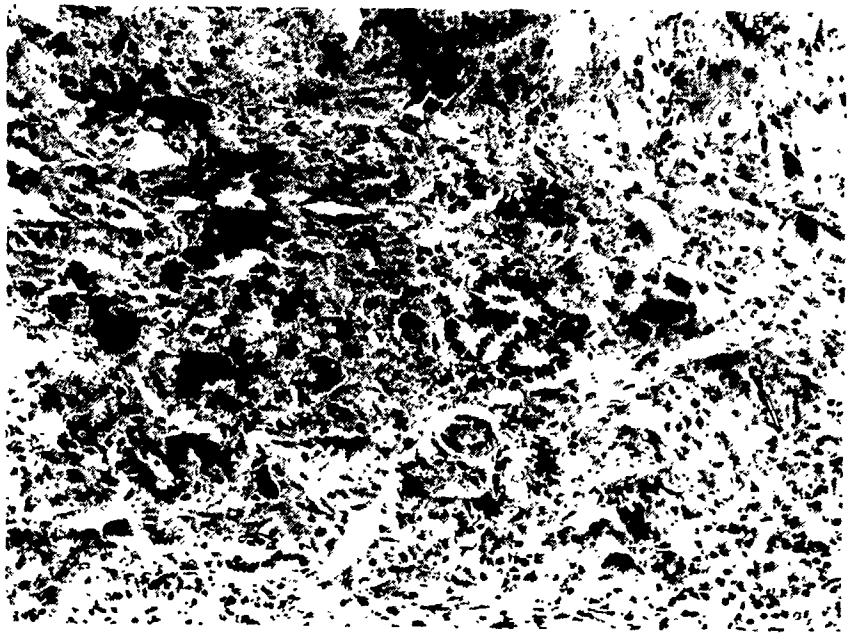
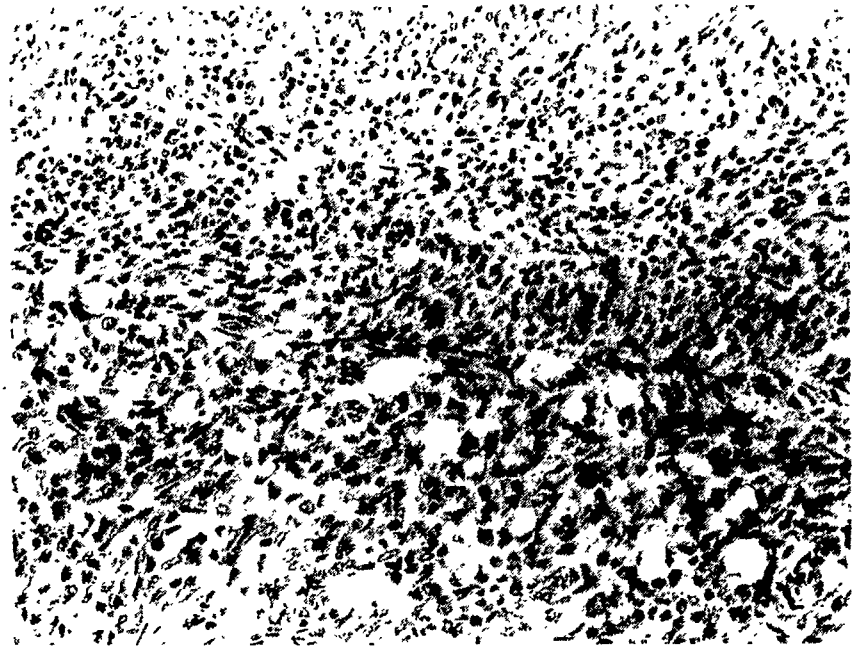


FIG. 6. FOCAL HYPERPLASIA AND PROLIFERATION OF EPITHELIUM-LIKE PLEURAL MESOTHELIAL CELLS IN AN ASBESTOS GRANULOMA FROM A MONKEY (× 150)



We differentiated benign and malignant pleural mesotheliomas: benign tumours included fibrous and adenoma-like tumours. We observed three types of malignant pleural mesotheliomas induced by asbestos in animals, i.e., carcinoma-like, sarcoma-like and mixed. Carcinoma-like mesotheliomas have solid, adenomatous, papillary and mixed structures; with regard to the latter, all variants of this type of tumour were observed. Sarcoma-like mesotheliomas are similar to certain fibroblastic sarcomas; they can comprise spindle cells and polymorphic cells. The central part of mixed mesotheliomas usually consists of sarcoma-like structures, and the peripheral part is represented by various kinds of carcinoma-like structures (Fig. 7). Such tumours give the most vivid and clearest evidence of the multipotential character of mesothelium cells, i.e., their ability to transform in various ways.

FIG. 7. MIXED PLEURAL MESOTHELIOMA INDUCED BY ASBESTOS IN A RAT (× 150)

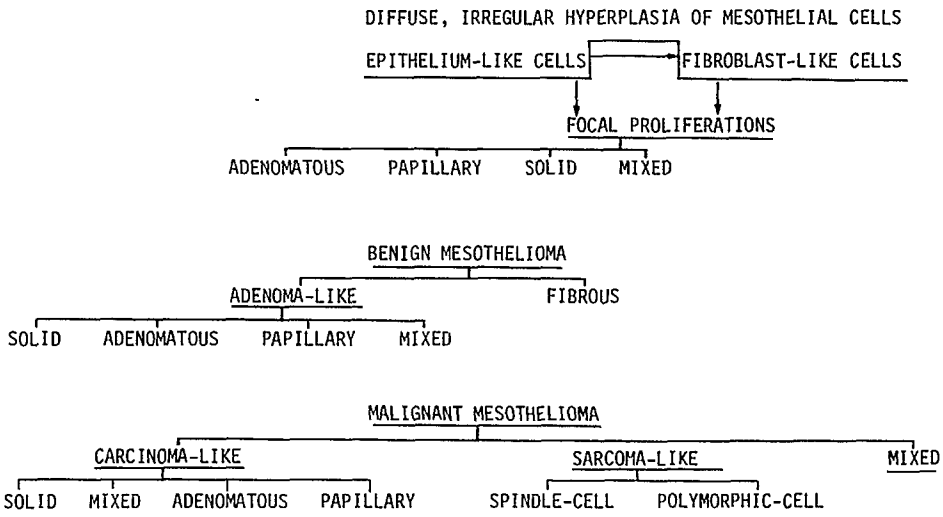


The detection of hyaluronic acid in both the cell cytoplasm and in the intercellular vacuoles usually found in tumours is an important criterion for the mesothelial origin of the tumours. Secretion of hyaluronic acid has been observed in 86% of the pleural mesotheliomas studied.

The morphological picture of these experimental pleural mesotheliomas is similar to that of analogous tumours in humans. This permits us to conclude that the mechanism of the action of asbestos on the pleural mesothelium in rats and hamsters is probably similar to that in humans.

No unified morphological classification of mesothelial tumours in humans has been elaborated so far. We have proposed a histological classification of experimental pleural mesotheliomas (Fig. 8) which, in our opinion, might be used for the development of a classification of such tumours in humans.

FIG. 8. HISTOLOGICAL CLASSIFICATION OF PREMESOTHELIOMATOUS LESIONS AND PLEURAL MESOTHELIOMAS INDUCED BY ASBESTOS IN RATS



SUMMARY

Asbestos samples were injected into the right pleural cavity of random-bred rats (20 mg thrice monthly). Milled commercial chrysotile induced pleural mesotheliomas in 65.5%, its dust in 46.3%, milled synthetic chrysotile in 2.4%, milled commercial magnesia-arphvedsonite in 77.1%, milled commercial anthophyllite in 41.4%, its dust in 64.9% and milled synthetic hydroxyamphibole in 54.5%.

Intratracheal injections (10 mg twice monthly) of milled commercial chrysotile into Syrian golden hamsters induced lung tumours in 63% (malignant in 44.4%, benign in 18.5%), synthetic chrysotile in 23% (malignant in 7.7%, benign in 15.3%), commercial magnesia-arphvedsonite (5 mg twice monthly) in 80.8% (malignant in 46.2%, benign in 34.6%) and synthetic hydroxyamphibole (5 mg twice monthly) in 63.4% (malignant in 19.2%, benign in 44.2%).

Benzo[*a*]pyrene adsorbed or added to asbestos dust stimulated asbestos-induced lung carcinogenesis in rats but had no influence on pleural carcinogenesis. Pretumorous lesions were found in lung tissue and in pleural mesothelium in all experiments in rats, hamsters and monkeys after instillations of asbestos into the lungs and pleural cavity.

The morphological picture of the pretumorous lesions and tumours was similar in all experiments. Diffuse and focal hyperplasia and proliferation of epithelium-like and fibroblast-like mesothelial cells were observed. Benign (adenoma-like, fibrous) and malignant (carcinoma-like, sarcoma-like, mixed) mesotheliomas were also found.

RESUME

Des échantillons d'amiante ont été injectés dans la cavité pleurale droite de rats exogames (20 mg trois fois par mois). Le chrysotile commercial broyé a provoqué des mésothéliomes pleuraux chez 65,5% des animaux, ses poussières chez 46,3%, le chrysotile synthétique broyé chez 2,4%, la magnésie-arphvedsonite commerciale broyée chez 77,1%, l'anthophyllite commerciale broyée chez 41,4%, ses poussières chez

64,9% et l'hydroxy-amphibole synthétique broyée chez 54,5%.

Le chrysotile commercial broyé injecté par voie intratrachéale (10 mg deux fois par mois) à des hamsters dorés de Syrie a induit des tumeurs pulmonaires chez 63% des animaux (malignes: 44,4%, bénignes: 18,5%), le chrysotile synthétique chez 23% (malignes: 7,7%, bénignes: 15,3%), la magnésiearphvedsonite commerciale (5 mg deux fois par mois) chez 80,8% (malignes: 46,2%, bénignes: 34,6%) et l'hydroxyamphibole synthétique (5 mg deux fois par mois) chez 63,4% (malignes: 19,2%, bénignes: 44,2%).

Le benzo[*a*]pyrène adsorbé ou ajouté aux poussières d'amiante stimulait la cancérogenèse pulmonaire induite par l'amiante chez le rat mais il n'influait pas sur la cancérogenèse pleurale. Des lésions prétumorales ont été observées dans le tissu pulmonaire et le mésothélium pleural lors de toutes les expériences effectuées sur le rat, le hamster et le singe après instillation d'amiante dans les poumons et la cavité pleurale.

Le tableau morphologique des lésions prétumorales et des tumeurs était identique dans toutes les expériences. On a constaté une hyperplasie diffuse et focale et une prolifération de cellules mésothéliales épithélioïdes et de type fibroblastique. Ont été également observés des mésothéliomes bénins (de type adénome, fibreux) et malins (de type épithéliome, de type sarcome, mixtes).

REFERENCES

Cunningham, H.M. & Pontefract, R.D. (1971) Asbestos fibers in beverages and drinking water. *Nature, 232*, 332-333

Nicholson, W.J. & Pundsack, F.L. (1973) *Asbestos in the environment.* In: Bogovski, P., Gilson, J.C., Timbrell, V. & Wagner, J.C., eds, *Biological Effects of Asbestos (IARC Scientific Publications No. 8)*, Lyon, International Agency for Research on Cancer, pp. 126-130

Pylev, L.N. (1972) Morphological lesions in rats lungs, induced by intratracheal injection of chrysotile-asbestos alone and with benz-[*a*]pyrene. *Vop. Oncol., 18*, 40-45

Sébastien, P., Bignon, J., Gaudichet, A., Dufour, G. & Bonnaud, G. (1976) Les pollutions atmosphériques urbaines par l'asbeste. *Rev. fr. Mal. resp., 4, Suppl. 2*, 51-62

Selikoff, I.J. & Hammond, E.C. (1978) Asbestos-associated disease in United States shipyards. *Cancer, 28*, 87-99

Wagner, J.C. (1970) *The pathogenesis of tumours following the intrapleural injection of asbestos and silica.* In: Nettesheim, P., Hanna, M.G. & Deatherage, J.W., eds, *Morphology of Experimental Respiratory Carcinogenesis*, Springfield, VA, National Technical Information Service, pp. 347-354

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