combustion were soot and unburned fuel. These may also explain the lower rate of lung cancer in RS.

Thus, to date, the collaborative studies of Xuan Wei lung cancer have shown consistency among epidemiologic, physical, chemical, and toxicologic findings. The accumulating data increasingly suggest an etiologic link between indoor smoky coal burning and lung cancer.

Note added in proof: In our recent mouse skin tumor initiation promotion studies in female SENCAR mice using the organic extract as the tumor initiator and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) as the promoter, the organic extract of the smoky coal sample induced more papillomas per mouse at the dose of 10 mg per mouse (7.5 papillomas after 26 weeks of promotion) than the extract of the wood sample (2.1 papillomas). When applied dermally twice weekly (total 2 mg per mouse per week) to the mice without TPA promotion, the smoky coal sample induced carcinomas in 35% of the treated animals while no carcinomas were observed in the wood sample–treated mice or in the solvent control-treated mice after 52 weeks of the treatment (*13*).

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Preferred Microtubules for Vesicle Transport in Lobster Axons

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The hypothesis that transported vesicles are preferentially associated with a subclass of microtubules has been tested in lobster axons. A cold block was used to collect moving vesicles in these axons; this treatment caused the vesicles to accumulate in files along some of the microtubules. Quantitative analysis of the number of vesicles associated with microtubule segments indicated that lobster axons have two distinct populations of microtubules—transport microtubules that are the preferred substrates for vesicle transport and architectural microtubules that contribute to axonal structure.

MICROTUBULES ARE THE LINEAR substrates for vesicle transport in axons (*1–3*); in addition to their role in vesicle transport, they are essential structures in axonal architecture (*4*). These two functions—transport and architecture—place different demands upon the microtubules. In some cases, the requirements of architecture actually appear opposite to those of vesicle transport. For example, axonal microtubules have an extensive system of sidearms that contribute to the architecture of the axonal cytoskeleton by linking the microtubules to neighboring cytoskeletal structures (*5*). Although such cross-links help to define axonal architecture, they offer potential resistance to the movement of transported vesicles along the microtubules (*6*).

These and other differences between the requirements of vesicle transport and architecture may have fostered the evolution of two classes of microtubules—one class that is the preferential substrate for vesicle transport and another class that contributes primarily to axonal architecture. To examine the possibility that axons contain a subclass of microtubules that are the preferred substrates for vesicle transport, we analyzed the distribution of transported vesicles in lobster axons. In lobster axons, microtubules are the only long polymers (*7*). These axons, and those of other arthropods, lack neurofilaments; arthropods may have lost the genes for neurofilament proteins when the arthropod lineage diverged from the other metazoan phyla (*4, 8*). In arthropod axons, microtubules fulfill all of the architectural and transport functions required of cytoskeletal polymers in axons.

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To determine whether transported vesicles moved preferentially along particular microtubules in lobster axons, we used the cold-block method to collect and distinguish transported vesicles. A small region of the axon is cooled to 2° to 4°C (*3, 9, 10*). When moving vesicles reach the cold block, they stop and accumulate along the normal pathways of transport (*3, 9, 10*). In vertebrate and molluscan axons (both of which contain neurofilaments), the transported vesicles accumulate in files along microtubule domains, which are surrounded by neurofilaments; these microtubule domains are the

Table 1. Vesicle distribution among lobster axon microtubules assuming one homogeneous population of microtubules. Total vesicles, 346; average vesicles per microtubule, 1.18.

Vesicles per microtubule	Microtubules (number)	
	Observed*	Poisson prediction (m = 1.18)
0	212	90
1	14	106
2	11	63
3	9	25
4	10	7
5	12	2
6	8	0
7	8	0
8	4	0
9+	5	0
Total	293	293

*Observed and expected values are statistically distinct by both the chi-square test and the Kolmogorov-Smirnov test ($P < 0.01$).

corridors for intraaxonal vesicle transport (*3, 10, 11*).

Like the axons of other arthropods, lobster axons do not contain neurofilaments; thus, the microtubules are not segregated into corridors but are distributed rather uniformly in these axons (*7, 12, 13*). Nonetheless, if some of the microtubules in the lobster axons are preferred pathways for vesicle transport, vesicles should form files along only certain microtubules at a cold block. Alternatively, if all of the microtubules are equally capable of supporting vesicle transport, the vesicles should be distributed randomly and uniformly among the microtubules.

Vesicles accumulated proximal to the cold block within lobster axons (Fig. 1). Vesicles moving by retrograde transport also accumulated distal to the cold block, but these were a more heterogenous population, and because there were fewer of these than the vesicles moving by anterograde transport, they were less suitable for subsequent analysis. In control axons, almost all of the microtubules had no associated vesicles; a few microtubules had some vesicles associated with them.

The cold-block paradigm experimentally increases the number of transported vesicles that can associate with microtubules in the region adjacent to the cold block. Most transported vesicles were associated with only one or a few microtubules, and, in those axons that contained many vesicles, the vesicles were often aligned along a single microtubule (Fig. 1, B and C). These vesicles were very tightly packed along the microtubules, and, in some cases, they were arranged helically around the microtubule (Fig. 1C). The association of the vesicles with only some of the many available microtubules—even when the amount of vesicles was increased by more than an order of magnitude above control levels—suggested that the vesicles were preferentially attached to certain microtubules.

As in the lobster axons, vesicles may also bind preferentially to certain microtubules in vertebrates. For instance, Smith (*14*) found that vesicles were closely associated with certain microtubules near the presynaptic terminal in larval lamprey axons; in some cases, the vesicles were also arranged in a helical pattern around a microtubule in these axons.

Our micrographs suggested that the vesicles were not randomly distributed among the microtubules. To test this impression, we counted the number of vesicles along 0.6-μm segments of microtubules. Along 293 microtubules, we found 346 vesicles; if these vesicles had been distributed randomly among all of the microtubule segments, then they should have approximated a single Poisson distribution. In the expected ran-

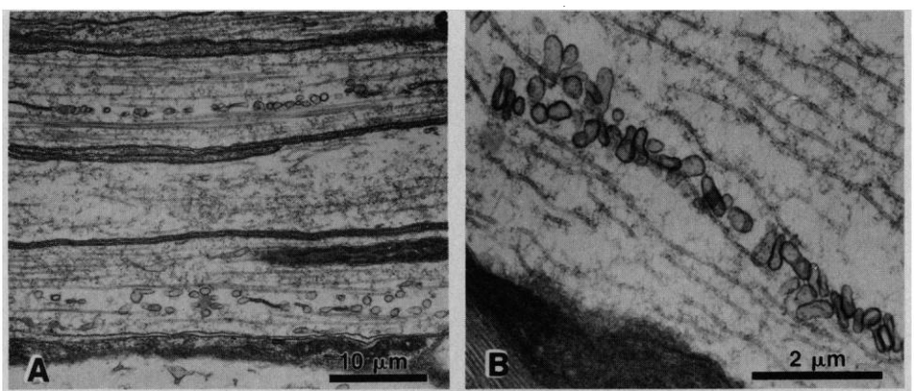

Fig. 1. Electron micrographs of longitudinal sections of axons on the anterograde side of the cold block. (**A**) At low magnification, small clear vesicles and membranous tubules are arranged in files that are parallel to the microtubules. (**B**) Round and oblong vesicles are closely associated with one microtubule. (**C**) The vesicles are very closely associated with the microtubules. In all of the experiments, 2- to 3-cm segments of nerve from the walking legs of lobsters were placed on a cold block in filtered seawater. The axons were subjected to cold block for 15 to 30 minutes and then rewarmed for 3 to 5 minutes prior to fixation. This method concentrates large numbers of vesicles that were being transported in an anterograde or retrograde direction for ultrastructural observations (*3*). In lobster, as in other axons, the morphology of the accumulated vesicles on the two sides of the cold block is different (*3, 9–11*). Anterograde vesicles are predominantly small-diameter, single-membrane vesicles and discrete tubular vesicular components that are separate and distinct from the axonal smooth endoplasmic reticulum. Vesicles that accumulate by retrograde transport are a more heterogenous population of larger complex organelles that includes multivesicular and dense core bodies. Both anterograde and retrograde vesicles accumulate in regions proximal to the cold block. Video-enhanced microscopic observations of these cold-blocked preparations show that, when rewarmed, the accumulated vesicles resume their normal movement along the microtubules. Furthermore, electron microscopic analyses of the transported vesicles indicate that the cold block–rewarming method preserves the normal relationship between the vesicles and their substrate microtubules (*3*). For electron microscopy, the nerves were fixed with 4% glutaraldehyde in EGTA-phosphate buffer with an osmolarity of 1200 mOsm, dehydrated, and then embedded in Poly Bed 812 resin (*3*). The accumulated vesicles were located by cutting through the center of the cold-blocked region and measuring to the known area of vesicle accumulation (*3*). To quantify the vesicle-microtubule association, we located 0.6-μm segments of microtubules in longitudinal sections of axons at a magnification of 10,000. Only the proximal 0.6-μm segment was analyzed in microtubule profiles that were longer than 0.6 μm. Vesicles were considered to be associated with a microtubule segment if they were within approximately 20 nm of that microtubule.

Table 2. Vesicle distribution among lobster axon microtubules assuming two homogeneous populations of microtubules. The low affinity microtubules constituted one-fourth of the total; the high affinity microtubules constituted three-fourths of the total.

Low affinity microtubules; total vesicles, 16; average vesicles per microtubule, 0.07

Vesicles per microtubule	Microtubules (number)	
	Observed*	Poisson prediction ($m = 0.07$)
0	211	209
1	10	15
2	3	0
3+	0	0
Total	224	224

High affinity microtubules; total vesicles, 330; average vesicles per microtubule, 4.78

Vesicles per microtubule	Microtubules (number)	
	Observed†	Poisson prediction ($m = 4.78$)
0	1	1
1	4	3
2	8	7
3	9	11
4	10	13
5	12	12
6	8	10
7	8	7
8	4	4
9+	5	1
Total	69	69

*Observed and expected values cannot be statistically distinguished by either the chi-square test ($P < 0.6$) or the Kolmogorov-Smirnov test ($P < 0.9$). †Observed and expected values cannot be statistically distinguished by either the chi-square test ($P < 0.7$) or the Kolmogorov-Smirnov test ($P < 0.9$).

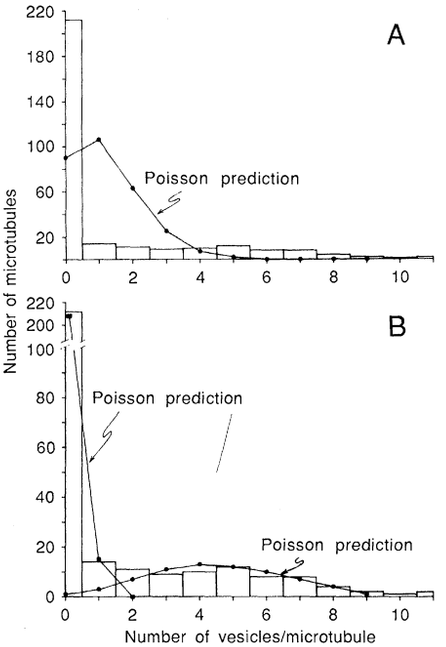

Fig. 2. Histograms showing the number of vesicles associated with 0.6-μm segments of microtubules that are located adjacent to a cold block. (**A**) The distribution of vesicle-microtubule associations is compared with the single Poisson distribution that is expected if the vesicles had been randomly distributed among all of the microtubules. The actual distribution differed significantly from the random distribution; notably, an inordinately large number of microtubules had no vesicles (Table 1). (**B**) The total distribution of vesicle-microtubule associations is divided into two subclasses that overlap in the 0, 1, and 2 vesicles per microtubule bins. It is assumed that each distribution tapers smoothly to zero in this overlapping range. The average number of vesicles per microtubule was counted for each subclass (Table 2) and predicted random (Poisson) distributions were calculated for each curve. The actual values were found to be statistically indistinguishable from the predicted Poisson values (Table 2). This suggests that the two subclasses of microtubules have significantly different affinities for transported vesicles, but that the affinities are fairly uniform within each of the two subclasses. One factor that could contribute to the breadth of the zero bin distribution in these experiments is the inability to distinguish occasional vesicles that are fortuitously adjacent to architectural microtubules from those that are effectively attached by vesicle transport crossbridges.

dom distribution, most 0.6-μm microtubule segments would have had one vesicle (Fig. 2A). However, we found that the overwhelming majority of the microtubule segments had no vesicles. The actual distribution differed significantly from the expected Poisson distribution (Table 1); notably, an inordinately large number of the microtubule segments we observed were completely bare.

This observation is consistent with the hypothesis that there are two distinct subclasses of microtubules in the lobster axons—one population (the architectural microtubules) that has a poor affinity for vesicles and a second population (the vesicle transport microtubules) that has a much greater affinity for vesicles. If this hypothesis is correct, then the total vesicle distribution should approximate the sum of two distinct Poisson distributions. In fact, the total vesicle distribution separated as two distinct populations (Fig. 2B) as predicted by this hypothesis; the actual observations are statistically indistinguishable from the values expected if one assumed random distribution of vesicles among two distinct populations of microtubules (Table 2). Moreover, these analyses suggest that, in lobster axons, one-fourth of the microtubules are transport structures and three-fourths of the microtubules are architectural structures.

The data are consistent with the assumption of two distinct Poisson populations within lobster axons in that the vesicles distinguish between two distinct subclasses of microtubules (architectural and transport) but do not distinguish among the microtubules within each class. Studies of the detailed mechanisms of vesicle transport suggest some of the mechanisms by which vesicles could distinguish between the two subclasses of microtubules: Vesicle transport in axons is mediated by crossbridges, which (in the squid giant axon) are 16 to 18 nm long (*3*) and in chick may have associated adenosinetriphosphatase (ATPase) activity (*15*). In lobster axons, the vesicle transport crossbridges also appear to be 16 to 18 nm long.

The dimensions of the crossbridges govern the distance between a transported vesicle and its substrate microtubule. Vesicles must be within 18 nm of a microtubule for the crossbridge mechanism to operate. Thus, any longer structures (such as the microtubule sidearms) that intervene between microtubules and vesicles can interfere with the crossbridge binding and the subsequent vesicle transport. In crustacean axons, the microtubules have an extensive system of 25-nm sidearms, which are attached to the microtubule surfaces and project from these surfaces (*12, 13*). Because they are located on the surface of the microtubules, these 25-nm sidearms may modulate the efficacy of vesicle transport by interfering with the attachment cycle of the 16- to 18-nm vesicle transport crossbridges. Through such effects on vesicle transport, an unequal distribution of 25-nm sidearms or other structures among the microtubules may determine the preference of the vesicles for the transport microtubules in lobster axons. Such accessory structures may then distinguish those microtubules that are largely architectural from those that are preferentially transport structures.

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