Allison and Nunn Revisited

To the Editor.—Allison and Nunn's 1968 hypothesis concerning the reversible depolymerization of microtubules as a possible mechanism of anesthesia has been challenged by Kennedy et al. and, more recently, by Saubermann and Gallagher, who noted that both pentobarbital and halothane failed to depolymerize microtubules in mouse optic nerve. These studies essentially confirm our experiments performed more than four years ago and first published in a letter to the editor of Lancet in 1970. The full results of our experiments, complete with oscilloscope tracings and electron micrographs, were subsequently published in 1974 and acknowledged in an editorial review in ANESTHESIOLOGY entitled "Neuronal Microtubular Systems." 

We recorded the ability of rabbit vagus nerves to conduct electrical impulses in vitro in the presence of 3 and 10 mM halothane, or 10 mM colchicine, a well-known microtubule-depolymerizing agent. Under these conditions, we found that 3 and 10 mM halothane blocked the ability of the nerve to conduct impulses within 25 and 10 minutes, respectively, but, surprisingly, yielded a significant increase in the number of microtubules per square micron of axonal area. Colchicine treatment reduced the microtubule population by 60 per cent but had an appreciable effect on the electrical activity of the nerves. We feel that our refutation of Allison and Nunn's hypothesis is, besides being the first, still the most experimentally complete, since we showed that: 1) halothane concentrations which quickly suspended action potential conduction led to an increase in microtubule numbers; and 2) colchicine treatment significantly reduced the formed microtubule population but did not affect the electrical activity. Thus, we showed by two methods that there was no correlation between electrical conduction and microtubular content and, therefore, that reversible microtubular depolymerization could not be the direct molecular basis of anesthesia.

Although the validity of the increase in microtubule population following halothane treatment may be questionable owing to the in vitro nature of the experiment, we believe that even when the widest likely margin of error is considered, there was certainly no decrease in the microtubule population of halothane-treated nerve segments. On the other hand, subtle chemical differences between tubulins (microtubule proteins) isolated from various sources have been well documented, as pointed out by Saubermann and Gallagher. Therefore, it is possible that the increase in microtubules which we observed may be a specific property of rabbit vagus tubulin, since crayfish axonal microtubules undergo a structural transformation when treated in a similar fashion. A more probable way to account for our observed increase in the microtubule population following halothane treatment is that halothane alters the axonal membrane with respect to inorganic ion permeability, which could, in turn, alter the axonal microenvironment and consequently shift the dynamic microtubule-subunit equilibrium in axons to favor the polymerized microtubule form (see Samson and Hinkley for a brief review). Anesthetics have long been thought to alter cellular membrane systems, and we have found that halothane concentrations higher than 10 mM cause extensive axonal membrane disruption and a concomitant disruption of axonal microtubules (unpublished data). Perhaps the concentrations of halothane we employed altered the axonal membrane in such a fashion as to create transitory microenvironments favoring microtubule polymerization. This possibility is entirely open to experimental testing by in-vitro studies similar to those reported by Schlaefer.

The last point to be made is one regarding the nomenclature used by Saubermann and Gallagher. In addition to analyzing the microtubule population of axons, they also attempted to quantify the "microfilament" population of the same axons and plotted microtubule-microfilament ratios. It is unclear whether they in fact mean microfilaments, or more likely, "neurofilaments," since no morphologic description of the organelles which they call "microfilaments" is given in the text and no electron micrographs are presented. Both neurofilaments and microfilaments are "fibrous organelles" found in neural tissue, but whereas neurofilaments seem to be confined in distribu-
tion to cells of neural origin, microfilaments are probably a universal component of all cell types. Moreover, there are obvious structural, important chemical, and implied functional differences between these two structures. Neurofilaments average 50–120 Å in diameter, are composed of a distinctive protein, and are easily identified in average electron micrographs. Microfilaments average about 50–70 Å in diameter and can be observed only in good-quality electron micrographs of favorably oriented sections. Even then, only faint individual microfilaments may be visible. In addition, microfilaments are now generally associated with intracellular translocation processes due to their composition of actin-like protein. Some clarification of terminology would be most helpful in interpreting the results obtained by Saubermann and Gallagher and would be extremely useful to those of us actively investigating the role of microfilaments in nerve cell function and the effect of anesthetics on microfilamentous structures.

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REFERENCES

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To the Editor.—Our paper does not absolutely refute the Allison–Nunn hypothesis, but rather detracts from it as a likely explanation of the mechanism of anesthesia. We failed to demonstrate a morphologic change in situ in an experiment in which clinical concentrations were used, allowing microtubule environmental concentration and morphology to be biologically determined. Dr. Hinkley showed that halothane in concentrations “higher than those used clinically” blocked the nerve’s ability to conduct an impulse. This was correlated with electron micrographs of a segment of nerve tissue incubated in vitro with halothane. The relevance of this finding to the mechanism of anesthesia remains unclear, since nerves do carry impulses in the intact animal during general anesthesia.

There is still considerable confusion in nomenclature applied to microtubules and their subunits. In fact, in a review on Neuronal Fibrous Proteins chaired by Drs. Schmitt and Samson, several different nomenclatures were used. We used the term “microfilament” to represent what Dr. Hinkley terms “neurofilament.” We chose this term to avoid confusion with the light microscopists’ “neurofibril.” A neurofilament is, according to one nomenclature, a form of microfilament. Others, however, have referred to protofilaments (40–90 Å) as microfilaments.

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