Interfacial models of nerve fiber cytoskeleton

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ABSTRACT A new approach, basing on a resemblance between cytoskeleton structures associated with plasma membranes and interfacial layers of coexisting phases, is proposed. In particular, a lattice model, similar to those of the theory of surface properties of pure liquids and nonelectrolyte solutions (Ono, S., and S. Kondo. 1960. Handbuch der Physik.), has been developed to describe nerve fiber cytoskeleton. The preliminary consideration of the model shows the existence of submembrane cytoskeleton having increased peripheral densities of microtubules (compared with the bulk density) which is in qualitative agreement with the data in literature. Some additional possibilities of the approach proposed are briefly discussed.

INTRODUCTION

Cytoskeleton is known to control many cellular activities, such as maintenance of cell shape, cell contact or cell-solid substrate interaction, intraaxonal transport, etc. This polyfunctionality of cytoskeleton probably determines the morphological variety of its organization within different cells. Biochemical and structural aspects of cytoskeleton have been intensively studied in the last decade (Grain, 1986; Svitkina et al., 1983; Kobayashi et al., 1986; Tsukita et al. 1986). However, as to the physical chemistry of cytoskeleton structures, relatively little is known on the subject.

Microtubules, neurofilaments, and microfilaments form various dense networks in cytoplasm, and, therefore, any general analysis of such structures is difficult. However, some unordered networks associated with plasma membranes, such as submembrane layer of actin filaments in amoeba cells, cortex layer of mammalian fibroblasts, nerve fiber cytoskeleton could be discussed in terms of physical chemistry. To explain this point the following considerations seem to be useful.

In the squid giant axon there exists a distribution of microtubular density along the distance from the axolemma, as can be seen from Fig. 1, reproduced from Sakai et al. (1985). This fact allows an assumption that some affinity to the membrane might be attributed to cytoskeleton blocks formed by microtubules and filaments, and its value exceeds the interaction of the blocks with each other. From the physical point of view, the situation is similar to that of an interfacial layer between two coexisting phases where distributions of components are also observed (Ono and Kondo, 1960; Rowlinson and Widom, 1982). Under usual conditions, the width of such a layer does not exceed ten molecular sizes which constitutes several nanometers for pure liquids and nonelectrolyte solutions. According to Sakai's results (Fig. 1), the range of variable microtubular density is ~2 μm. Therefore the "molecular size" of cytoskeleton (or the constant, λ_0, of the lattice formed by cytoskeleton elements) should be of the order of 0.2 μm if the analogy used above is correct. Because this value is comparable with the diameter of a microtubule (0.03 μm) the analogy proposed seems to be reasonable.

There also exist other experimental manifestations of the resemblance between the axon's cytoskeleton and interfacial systems. In a previous paper (Levin et al., 1988) we studied the dependence of the integral density of microtubules, D, on the effective radius, R_e, of the axons containing these microtubules. A cross-section of a nerve fiber bundle of *Carcinus maenas* leg is represented in Fig. 2. From these electron micrographs one can calculate both the total amount of microtubules within the axons and their cross-section area (πR_e^2). This allowed to find out that the dependence of interest can be expressed as

$$D(1/R_e) = a_0 + b_0/R_e, \tag{1}$$

which implies an increase of the microtubular density within thin axons compared with thick ones (a_0 and b_0 are certain positive constants). Because the result is consistent with the microtubular distribution pointed out above, we can proceed to more important conclusions.

In terms of local description the integral density, $D(1/R_e)$, can be determined as

$$D(1/R_e) = \frac{2}{R_e^2}\int_0^{R_e} C_{\text{int}} r\,dr, \tag{2}$$

where C_{int}, the local concentration of microtubules in an axon's cross-section, and r, the radius. Using the above expressions to eliminate density D, we find the integral equation for C_{int}:

$$a_0 R_e^2 + b_0 R_e = 2\int_0^{R_e} C_{\text{int}} r\,dr. \tag{3}$$

One can easily show that a solution of the equation cannot be a sole function of the distance from the axo-

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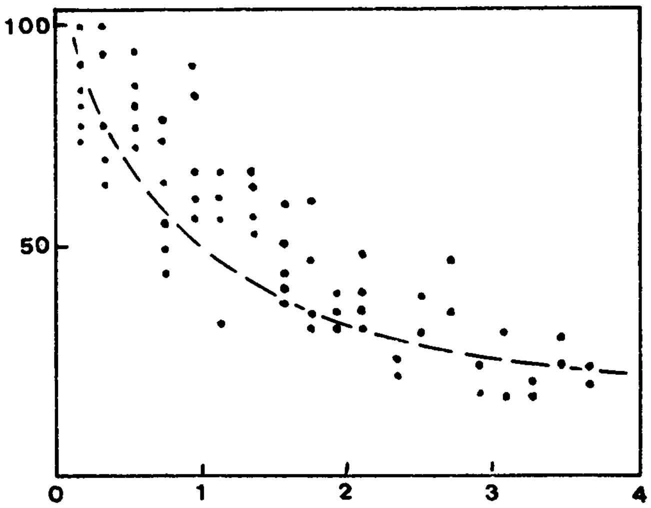

FIGURE 1 Number of microtubules in μm^2 of cross-sectional area as a function of distance from axolemma to the interior. (Sakai et al., 1985).

lemma, $z = R_e - r$, but should be also dependent of the radius, R_e, i.e., $C_{int} = f(R_e - r, R_e)$. Such behavior of C_{int} shows nonadditivity of the property at radius variations and is characteristic of problems of overlapping interfacial layers (for example, the double layer problem for two charged surfaces [Derjaguin and Duhin, 1976]). Thus, another manifestation of the initially assumed analogy is observed. Therefore, one may suggest that the cytoskeleton organization in nerve fibers results from the same physical reasons as those which are responsible for the existence of interfacial layers in simpler objects, for example, gas-liquid systems.

So, the main aim of the paper is to adopt the theory of interfacial layers for a description of cytoskeleton structures. As an initial step in quantitating cytoskeleton structures adjacent to a cellular membrane, we have reformulated the model which has been applied in the "hole" theory of pure liquids and nonelectrolyte solutions (Ono and Kondo, 1960). Given this background, we shall obtain a difference equation that describes the cytoskeleton density as a function of the distance from the membrane. Performing a redetermination of the quantities included in this equation, we shall then get its differential analogue that corresponds to the local treatment of free energy of a nonhomogeneous solution (Cahn and Hilliard, 1958). We will also compare the results obtained with those which could be derived in the case of systems formed by small particles. Further, we will calculate the cytoskeleton density in the system of two planar membranes with the variable separation distance to model changes in integral density D at radius variations. Because the measurements mentioned above show that density D should trace out a linear dependence when plotted versus reciprocal axon's radius, we shall use this circumstance to choose parameters of the model proposed. Finally, we will discuss possible applications of the theory to biological objects and processes. We will divide our account in two parts. In the present paper, the main attention will be devoted to physical motivations for choosing an adequate cytoskeleton model and to the development of a special method for acquiring the necessary quantitative results. The remainder of the program outlined above will be considered in the next paper.

GLOSSARY OF PRINCIPAL SYMBOLS

A	Cross-section area of the volume considered (continuous approach)
c	Lattice coordination number (4 or 2 in present paper)
C_{int}	Local concentration of microtubules in an axon's cross-section
$D(1/R_e)$	Integral density of microtubules in an axon's cross-section
D	Diffusion coefficient
d_0	Persistence length of a cytoskeleton quasiparticle (unit)
e	Base of natural logarithms
F, H	Free energy, enthalpy
$\bar{F}_c, \bar{H}_c$	Free energy, enthalpy of a cytoskeleton lattice per its one segment of length d_0
$\bar{f}_c^{(i)}, \bar{h}_c$	Free energy, enthalpy of the ith layer of the lattice (per segment)
i, j	Ordinal numbers of lattice layers or particles
K	Number of uniformly spaced layers within the cytoskeleton lattice
k	Boltzmann constant
L	Length of a microtubule from an origin
L_0	Total length of a microtubule
L_l	Correlation radius in the longitudinal direction
L_x	Correlation radius for the normal (to a membrane) and latitudinal directions
M	Total number of layers in the cytoskeleton lattice
m	Number of cytoskeleton units within a single layer which suffer bendings under the transition from a preceding segment (lth in the paper) of the system to the subsequent ($[l + 1]$-th) one
m_j	Number of ways in which the jth unit can be continued at the transition indicated above
N	Total number of sites (cells) within a single layer of the lattice
n_i	Number of sites occupied by cytoskeleton units within the ith layer
P_q	Probability of finding a central quasiparticle having q particles of the different kind and $(c - q)$ particles of the same kind as nearest neighbors. In the paper, such statistical combinations are called q-configurations of a central particle.
$P_q^{(p)}$	Probability of finding p nonblocked vacancies among q ones of the q-configuration chosen
R_e	Effective radius of the axon chosen (in electron micrographs)

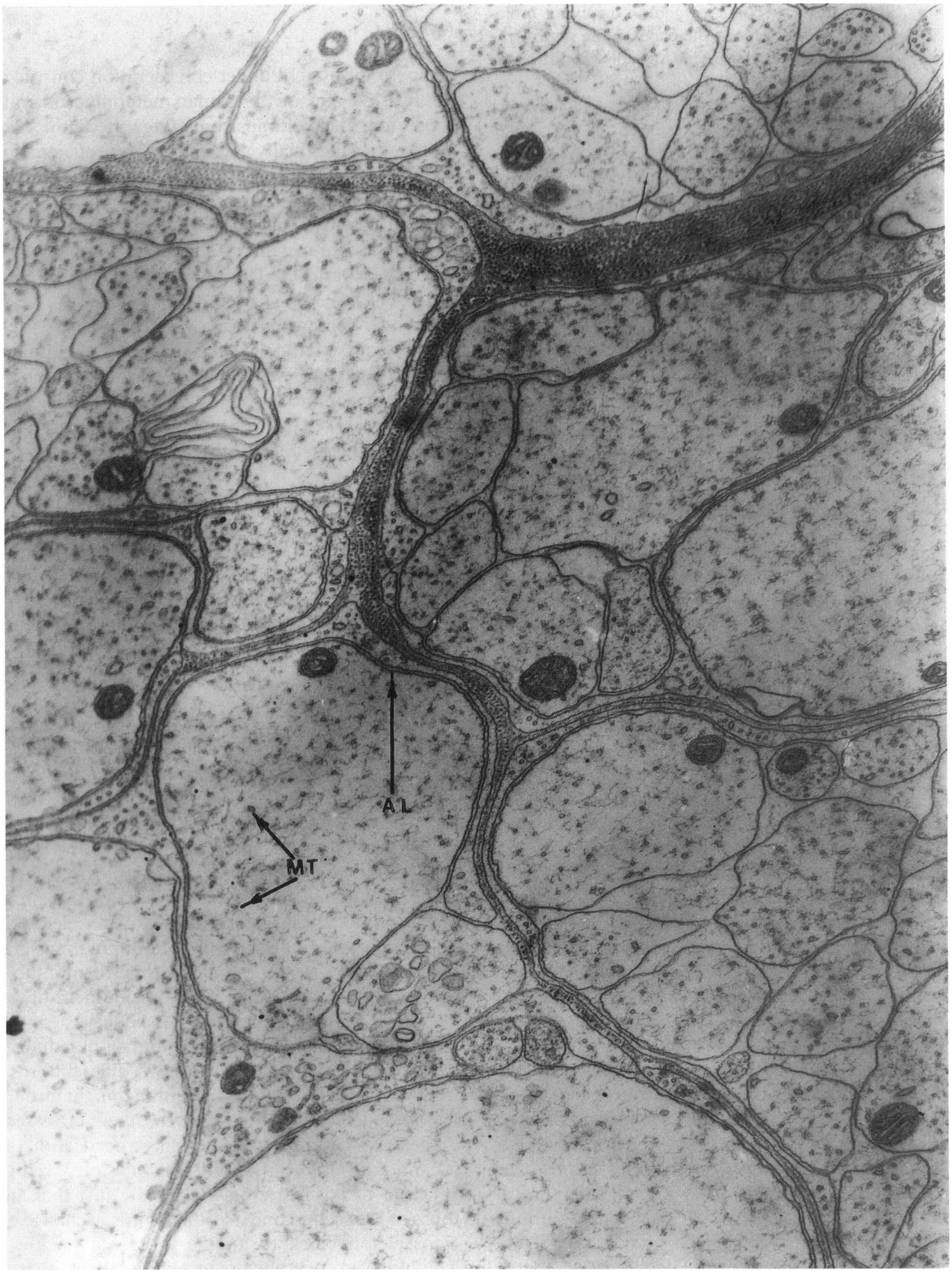

FIGURE 2 Cross-section of a nerve bundle of *Carcinus maenas* leg. Al, axolemma; Mt, microtubule.

r	Total number of chain segments of a polymer molecule (a microtubule in the paper). The same symbol is used to denote radial variable (in Introduction only).
$S, \bar{S}$	Entropy, the same property per one segment
T	Absolute temperature
t	Time
u_0	Bond energy of a cytoskeleton unit per length d_0
$W, \bar{W}_c$	Number of ways of arranging a system from its constituents, the same property for one segment of the system in question
ϵ_{ij}	Corresponding energy for the interaction of component i with j in a multicomponent system
λ_0	Lattice constant (size of a lattice cell)
μ	Chemical potential of cytoskeleton particles
μ_β	Energy per one bending of a unit
Π	Osmotic pressure

THEORY

Different molecular approaches are used in the physical chemistry of pure liquids and nonelectrolyte solutions to describe their surface properties and the structure of the arising interfacial layers. According to that elaborated by Ono and Kondo (1960), the system of two coexisting phases (for example, a liquid and its vapor) is modeled by considering a multilayered structure in between the interface and the bulk phase. The separation distance between the neighboring layers is put equal to the molecular size of liquid particles, so that some modification of the "hole" theory of liquids is used with this approach. It is also assumed that the local density of the phase considered varies through the first few layers and, probably, attains some bulk value at large distances from the interface. To seek a suitable distribution of the density, a minimization of the free energy of the system is carried out provided that the total amount of molecules within the volume considered is a constant. There are two factors that might contribute to the free energy of such nonhomogeneous system. One is a change of entropy due to the loss of possible sites for a distribution of molecules with the decrease in the distance from the interface and, correspondingly, the increase in the number of holes. The other is an enthalpy change accompanying variations in the composition because the interactions between holes and molecules are supposed to be different. A calculation of both factors (and, thereby, free energy) is a relatively simple problem for the case of nonpolar liquids where only short-range interactions can be considered, but the problem becomes difficult in a general case. Difficulties also appear for polymer solutions which might be, in a sense, likened to cytoskeleton structures surrounded by cytoplasm. Therefore, the use of some simplified models of cytoskeleton seems to be appropriate to provide some quantitative knowledge of cytoskeleton organization.

MODEL

Suppose the system under consideration consists of the volume, V, and of the planar membrane of unit area separating the volume from outer medium. In other words, we will initially neglect curvature effects. Volume V can be divided by surfaces parallel to the membrane into M consecutive layers so that the separation distance between the dividing surfaces would be constant and equal to λ_0, for instance. A similar layout of the volume can also be performed for the other (longitudinal) direction using $(N + 1)$ surfaces perpendicular to the membrane and the same separation λ_0. As a result, there appears a square lattice of sites at any cross-section of the system. For the present analysis we shall assume the intracellular volume to consist of only two material species; the cytoskeleton units including a microtubule with adjacent intermediate filaments (and, probably, some amount of neighboring neurofilaments), and a second species consisting of the aqueous solution that fills the rest of the volume. We also assume that any site of the lattice introduced can be occupied with one and only one quasiparticle out of two kinds indicated. It means that the composition in a cross-section of every ith layer parallel to the membrane is determined by the number of its sites, n_i, from the total amount N which are occupied by particles of the first kind, for instance. It is natural to think that this composition remains constant along the length of the layer, provided N is large enough. In view of the facts indicated below, we were inclined to assume that a random character of the cytoskeleton lattice ensures this constancy. Actually, it follows from Fig. 1 that the values of the density of microtubules at a given distance from the axolemma are scattered within some range, and, therefore, only a large sample from such data would provide a reliable mean value of this property. Thus, it should be thought that some cytoskeleton blocks of different microtubular density are randomly distributed at a cross-section parallel to the membrane. This creates premises for a probabilistic treatment of the lattice under consideration. For it can be suggested that any microtubule has an arbitrary number of nonoriented bendings so that its position at two different cross-sections perpendicular to the membrane might also be different. When the distance between these cross-sections exceeds some value L_1 called usually a correlation radius, it cannot be claimed that microtubules observed in the ith layer of the first cross-section are just the same as those of the second one. In other words, a change of one cytoskeleton block to another occurs along the fiber length so that each one organized mainly by quasiparticles not belonging to the preceding block, might have different content of microtubules. Obviously, the same could refer to both the latitudinal and normal (to the

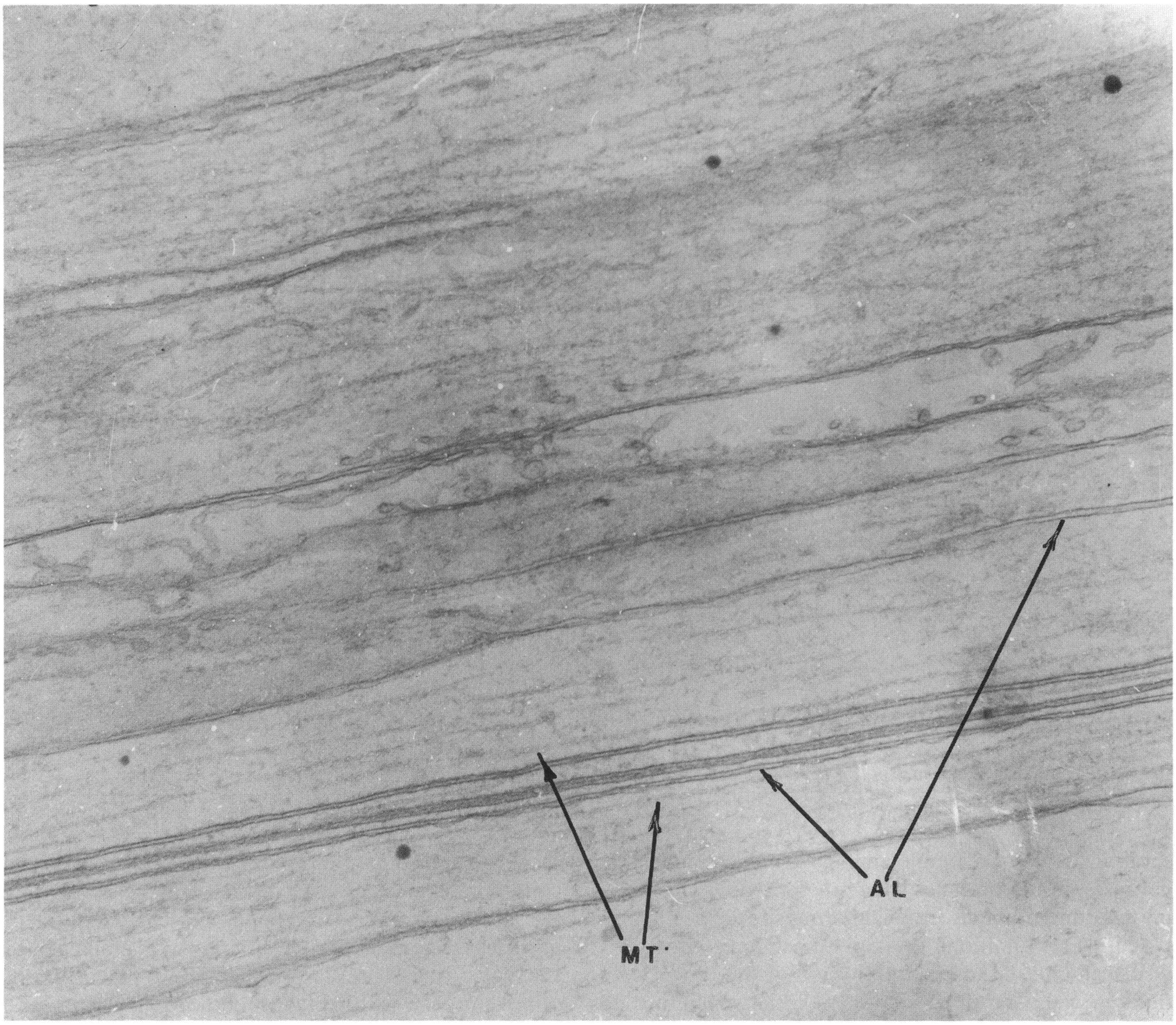

FIGURE 3 Longitudinal section of a nerve bundle of *Carcinus maenas* leg. Al, axolemma; Mt, microtubule.

membrane) directions with the only difference that suitable correlation radius, L_x, is to be of a smaller value than L_1, because microtubules are preferentially oriented in the longitudinal direction as the observations show (see Fig. 3). So, one can assume that bendings distributed along the length of microtubules are responsible for the observed macroscopic fluctuations in the cytoskeleton density. Actually, one can see from Fig. 3 that only at first sight the microtubules seem to be parallel to the axolemma; the probability of their bendings is higher the larger the diameter of the axon. It is also reflected by the fact that microtubules have a chaotic distribution in the cross-sections of thick axons (see Fig. 2) and form some structure looking like a square lattice in the case of thin axons, as seen in Fig. 4.

To take into account the peculiarities of microtubules pointed out above, we shall further assume that any microtubule preserves its parallel orientation to the membrane for distances along the longitudinal direction being smaller than a certain value $d_0 \geq \lambda_0$. At distance d_0 from an origin a microtubule might retain its site in the lattice introduced or instantly go over to a neighboring site given the latter is not occupied by another microtubule or will not be. After this, the microtubule is again parallel to the membrane and can undergo the next bending at twofold distance $2d_0$, and so on. Thus, a cytoskeleton quasiparticle may be considered to consist of r chain segments, each of which has the length d_0 being a priori much larger than the size of a solvent molecule. Such a model is often applied in the theory of polymer

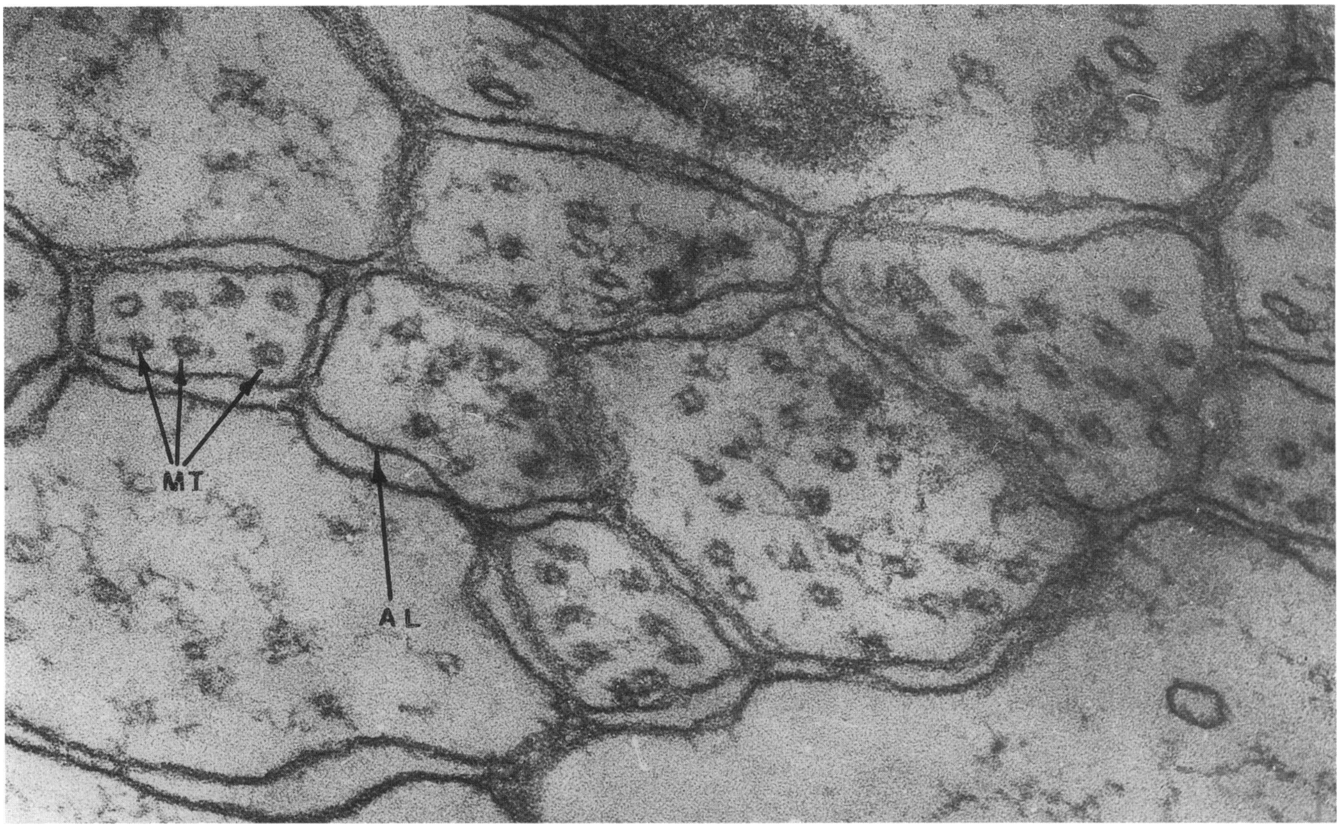

FIGURE 4 Cross section of thin axons of *Carcinus maenas* leg. Al, axolemma; Mt, microtubule.

solutions and has a simple analogy in the case of small densities of microtubules where the probability of finding two microtubules in neighboring sites is negligible. Under these conditions, one can expect that consecutive displacements of the microtubule axis will be given by a random walk trajectory or the well-known picture of Brownian diffusion. This picture will become complicated at arbitrary densities of microtubules where possible blocking of vacancies (holes) should be taken into consideration. Analysis of such a blocking effect will be the subject of further development. It will be clear from the following that the main problem is to calculate the configuration entropy of the polymer solution arising from the variety of ways of arranging the polymer and solvent quasiparticles within the volume considered. To arrive at a proper solution of the problem, we could use the approaches developed in the physics of polymers. According to Flory's method (Flory, 1953), one must consider an introducing the $(j + 1)$-th polymer molecule in the lattice volume provided that j molecules have been inserted previously at random. It is then necessary to calculate the number of ways in which the first segment of the $(j + 1)$-th molecule may be arranged in vacant cells of the lattice. After that, the similar estimation should be performed for the second segment of the same molecule and so on up to the last one (rth). An evident condition of such a consideration is a large extension of the lattice volume as compared with the length of the particles inserted that provides a proper averaging of the calculated quantity. We will here apply practically the same approach with certain modifications which result from peculiarities of the system in question. As far as we know, there are no systematic data on the length of microtubules in vivo at all, and in nerve fibers in particular. Therefore, the averaging required might be only based on the presumably large amount of sites N within a single layer of the lattice introduced. Under these conditions, it is reasonable to suppose the length L_0 of cytoskeleton quasiparticles to be practically infinite so that averaged distribution of quasiparticles will be then repeatedly realized along their length in the longitudinal direction. In this case, we may not consider continuations of all the consecutive segments of the particle chosen, but restrict our treatment only with transition from a certain intermediate segment $l \epsilon (1, r)$ of the particle to the next one, $(l + 1)$th. As a result, some modified procedure which differs from the one elaborated by Flory might be formulated. Its initial state which is taken as a pure solvent in the scope of Flory's method should be readdressed to the lth cross-section (or simply, segment) of the system where the distribution of the particles contained might be considered as their given configuration describing in terms of the mean probabilities of finding units and holes at arbitrary sites of the lattice. It is quite natural to assume such a configuration to be statistically homogeneous within the range of a given layer, because the similar approximation is usually applied to derive the enthalpy term, $\bar{H}$, of the free energy, $\bar{F}_c = \bar{H} - T\bar{S}$, per one segment of the system. Therefore, a summation of the pairwise interactions between nearest neighbors (i.e., the Bragg-Williams approximation) will be also used for this purpose. At the same time, a calculation of the entropy, $\bar{S}$, will be performed in a different manner than it is carried out usually. It follows according to the Boltzmann relation,

$$S = k \ln W, \tag{4}$$

that the entropy, S, should be determined by the total number of ways, W, in which the whole system might be organized from its constituents (k, the Boltzmann constant). In accordance with the supposition made above, we can assume the total entropy of the system, consisting of r single segments, to be the sum of r terms of the same value, $\bar{S}$, which is obviously the mean value of the entropy per one segment of the system: $S = r\bar{S}$. In other words, the requirement of a large length of cytoskeleton units is reformulated as the realization of mean properties of the system along the length L_0 of particles. This allows to restrict the further treatment with finding some mean properties and, as a result, to consider only a one-stepped process of continuing quasiparticles from a preceding segment of the system to the subsequent one instead of the r-stepped process of Flory's method. The principal feature of the procedure outlined is the necessity to take into account the environment of the particles continued since their distribution (and, hence, the environment) in the initial state (i.e., within the lth segment) is given by its statistical homogeneity. There also arise other features inherent in the approach applied, but they are only due to mathematical details of calculations. It is convenient to initially simplify the problem by supposing a homogeneity of the structure considered. In other words, we will assume (and this will be further proved) that a uniform array of the multilayered lattice in question arises far from the membrane. As an additional simplification of the problem we shall also suppose here that the planes of microtubular bendings might be oriented only parallel to the membrane. With the conditions just stated, microtubules can not leave the layer containing them, so that the number of ways m_j, of their continuation from the preceding segment of the system to the subsequent one should not exceed the quantity 3. As will be clear from the following, such a supposition, facilitating the necessary analysis, is not fundamental and can be omitted in a general case (this will be done in the next paper).

FORMULATION OF PROBLEM AND DEFINITIONS

According to the model proposed we must describe mean properties of square lattice formed by bending particles of practically infinite length. A calculation of the free energy of a certain intermediate segment of uniform content is the first step of such a description. We consider the free energy $\bar{F}_c$ to be the sum of those of single layers, $\bar{f}_c$, parallel to the membrane

$$\bar{F}_c = K\bar{f}_c, \tag{5}$$

where K, the number of uniformly spaced layers within the segment chosen. If $n < N$ is the amount of sites of every layer which are occupied by cytoskeleton particles, the quantities n/N and $(1 - n/N)$ give the probabilities of finding microtubules and holes at an arbitrary site in the lattice, respectively. It is worthwhile to use the following representations:

$$n = n\left(1 - \frac{n}{N} + \frac{n}{N}\right)^2 = n \sum_{q=0}^{2} C_2^{(q)} \left(\frac{n}{N}\right)^{2-q} \left(1 - \frac{n}{N}\right)^q \tag{6}$$

and

$$\begin{aligned} N - n &= (N - n)\left(1 - \frac{n}{N} + \frac{n}{N}\right)^2 \\ &= (N - n) \sum_{q=0}^{2} C_2^{(q)} \left(1 - \frac{n}{N}\right)^{2-q} \left(\frac{n}{N}\right)^q, \end{aligned} \tag{6'}$$

for the total amounts of microtubules and holes within a single layer. The above expressions might be treated as the sums of different configurations each of which includes a central particle (a microtubule or a hole) and $2 - q \geq 0$ particles of the same kind as nearest neighbors. We will further use the term "q-configuration" to denote such statistical combinations. Besides, we shall call a hole to be "nonblocking" one if the number of microtubules in its q-configuration is <2, and use the term "blocking" hole in the case of $q = 2$. Because a limited number $p \leq q$ of holes among the total amount q for a given configuration might be nonblocking, we also introduce the term "pq-configuration" to distinguish such combinations. The necessity of these definitions results from the following reasons. Considering ways of continuing the lth segment of a microtubule to the $(l + 1)$-th one, we should take into account the possibility of blocking neighboring holes with other microtubules, as was pointed out above. A competition between microtubules for a hole would not appear if the latter happens to be nonblocking. On the contrary, the competition effect might be very significant for the opposite case of block-

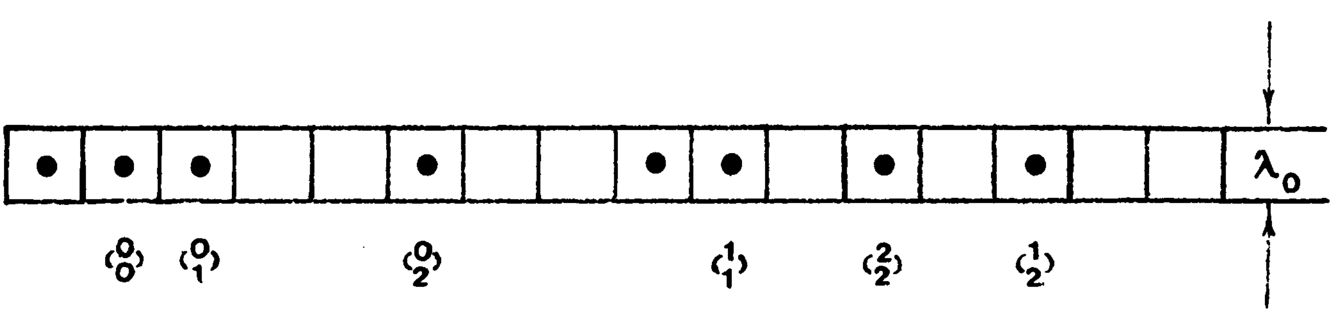

FIGURE 5 All the possible configurations of quasi-particles within a single layer of the lattice in question. Symbols $\binom{p}{q}$ correspond to *pq*-configurations of the respective quasi-particles marked by solid circles.

ing holes. To reflect such possibilities one can introduce the probabilities, $P_q^{(p)}$, of finding p nonblocking vacancies among the qs of the q-configuration chosen, so that Eq. 6 might be rewritten in the form:

$$n = n \sum_{q=0}^{2} C_2^{(q)} \left(\frac{n}{N}\right)^{2-q} \left(1 - \frac{n}{N}\right)^{q} \sum_{p=0}^{q} P_q^{(p)}, \tag{7}$$

where quantities $P_q^{(p)}$ satisfy obviously the relations:

$$\sum_{p=0}^{q} P_q^{(p)} = 1. \tag{8}$$

There are also three more relations for the probabilities introduced

$$P_1^{(1)} = 1 - \frac{n}{N}; \quad P_2^{(1)} = 2\frac{n}{N}\left(1 - \frac{n}{N}\right);$$
$$P_2^{(2)} = \left(1 - \frac{n}{N}\right)^2, \tag{9}$$

as one can easily establish. A reader can visualize the above definitions by using Fig. 5 which represents all the configurations introduced.

It is not out of place to explain additionally the use of Eqs. 6 and 7. Making use of these expressions, we thereby neglect a contribution of macroscopic fluctuations of the cytoskeleton density to the free energy. Really, it is reasonable to assume that the average distribution of quasiparticles given by Eqs. 6 and 7 yields the main contribution to mean properties of the system in question. Note that it is not necessary to introduce higher q-configurations of particles (with $q = 3, 4$), because transitions of microtubules from one layer to another are forbidden by the supposition made previously. This does not mean that a general consideration is extremely difficult; a complicated character of the corresponding results only forced us to simplify the problem. Besides, a subsequent analysis of an alternative case with the planes of bendings perpendicular to the membrane will allow a simple generalization of the consequences derived. Nevertheless, it should be pointed out that the existence of the higher q-configurations is taken into account in an implicit form because of the parabolic dependence of the enthalpy on the composition

$$\bar{h}_{BW} = \frac{cN}{2}\left[\epsilon_{11}\left(\frac{n}{N}\right)^2 + 2\epsilon_{12}\frac{n}{N}\left(1 - \frac{n}{N}\right) + \epsilon_{22}\left(1 - \frac{n}{N}\right)^2\right], \tag{10}$$

where $c = 4$, the coordination number; ϵ_{lk}, the energy of the pairwise interactions between particles l and k; and indices 1 and 2 are assigned to microtubules and holes, respectively. Beside this component, the bond energy of a microtubule is to be included into a proper expression for the enthalpy of a layer, $\bar{h} = \bar{H}/K$. Provided the parameter u_0 to be the bond energy of a cytoskeleton unit per length d_0, the expression

$$\bar{h}_0 = u_0\left(n + \frac{\lambda_0}{d_0} m\right) \tag{11}$$

is valid for the component discussed, where $m < n$, the number of microtubules possessing bendings within the segment considered. A similar component might be introduced for holes, but this would correspond to the addition of a certain constant into the resultant expression for $\bar{h}$ provided some redetermination of u_0 and the constancy of the total amount of sites, N. In this connection, it should be also emphasized that energy u_0 has a difference nature; it is to be independent of a zero energy point because there is an equilibrium between monomeric and polymeric species of the proteins forming a cytoskeleton lattice. As is common practice in the case of polymer solutions, we will not take into consideration the lower protein species, but their presence in the system is the reason for assigning the above matter to energy u_0. This means, in particular, that parameter u_0 should depend on the concentrations of monomeric proteins in a general case. A determination of such a dependence will not be solved here.

There is one more term to be included into the enthalpy of a separate layer:

$$\bar{h}_b = 2\mu_\beta m, \tag{12}$$

which reflects the bending energy of cytoskeleton units (μ_β, the energy per one bending, and factor 2 corresponds to the presence of bendings at both edges of the segment considered). Thus, the enthalpy

$$\begin{aligned}\bar{h} &= \bar{h}_{BW} + \bar{h}_0 + \bar{h}_b \\ &= \frac{cN}{2}\left[\epsilon_{11}\left(\frac{n}{N}\right)^2 + 2\epsilon_{12}\frac{n}{N}\left(1 - \frac{n}{N}\right) + \epsilon_{22}\left(1 - \frac{n}{N}\right)^2\right] \\ &\quad + u_0\left(n + \frac{\gamma_0}{d_0}m\right) + 2\mu_\beta m,\end{aligned} \tag{13}$$

can be calculated, provided the average number of bending units, m, is known. The latter is also necessary to calculate the entropy, $\bar{s} = \bar{S}/K$, of a separate layer. To do this, we shall use a special procedure for continuing microtubules from their lth segment to the $(l + 1)$-th one. In particular, we put the number of ways of such a continuation, $\bar{W}$, to be equal to

$$\bar{W} = \prod_{j=1}^{n} m_j \tag{14}$$

where $m_j \leq 3$, the number of ways of a continuation of the jth microtubule provided that $(j - 1)$ microtubules had been preliminary continued into the $(l + 1)$-th segment. Note definition (Eq. 14) of the statistical probability $\bar{W}$ differs from that used by Flory ($\bar{W}_F = (1/n!)\prod_{j=1}^{n} m_j$, in our symbols) because all the particles to be continued are fixed in the initial (lth) segment, and, hence, their rearrangement is impossible.

Obviously, the number of ways, m_j, depends on both the configuration of the microtubule chosen at the jth step (moment) of the continuation process and the probability of blocking the holes surrounding this particle. To take these factors into consideration, introduce the probability, $P_q(j)$, of finding a microtubule of the q-configuration and the degree of a hole blockage, $\lambda(j)$, at the jth moment of the process. In particular, define quantity $\lambda(j)$ to be the ratio of the number of holes which are blocked at the moment j within the $(l + 1)$-th segment to the total amount of the blocking holes, $(N - n)(n/N)^2$, in the lth one. In its meaning quantity $\lambda(j)$ is similar to Flory's "expectancy f_i that a cell" . . . (of the lattice) . . . "is occupied by a segment of a preceding molecule . . .", but differs from this probability due to the fact that only a blocking hole might be a blocked one. This leads to the impossibility to use Flory's approximation of "the average expectancy of occupation of a cell selected at random" for $\lambda(j)$ and requires a special consideration. Its necessary details are given in the Appendix.

With the definitions stated above, the partial number, $m_j^{(pq)}$, of continuations of a microtubule in the pq-configuration is given by the expression:

$$\begin{aligned}m_j^{(pq)} = P_q(j)P_q^{(p)}\sum_{i=0}^{q-p} C_{q-p}^{(i)}[\lambda(j)]^i \\ \times [1 - \lambda(j)]^{q-p-i}(q - i + 1),\end{aligned} \tag{15}$$

where $P_q(j)$ and $P_q^{(p)}$ are the probabilities mentioned previously; $C_{q-p}^{(i)}$ is the number of combinations of $(q - p)$ things i at a time which corresponds to an arbitrary blockage of i holes of $(q - p)$ blocking ones of the given pq-configuration; and $(q - i + 1)$, the possible number of bending continuations of the microtubule, $q - i$, plus the quantity 1 corresponding to the nonbending continuation. The total number, m_j, will be obtained after the summation of Eq. 15 over p and q.

Thus, the problem on the whole will be solved, in principle, if the quantities $P_q(j)$, $\lambda(j)$, m are known. As shown in the appendix to the present paper, all the above variables are determined in the same manner, which is the change of the numerical sequence $\{j\}$ by the continuous one where any values of j are admissible. In our opinion, the main features of the way applied will be clear after the following example. There is the well-known expression:

$$W = N!/n!(N - n)! \tag{16}$$

for the number of ways, W, of arrangement of n nondistinguishable particles among N sites. Reducing this relation as follows,

$$W = \frac{1}{n!}\prod_{j=1}^{n}[N - (j - 1)] = \prod_{j=1}^{n}\frac{[N - (j - 1)]}{j},$$

one can define the difference

$$\begin{aligned}\Delta S_i &= k\,ln\frac{W(i + 1)}{W(i)} \\ &= k\,ln\left\{\prod_{j=1}^{i+1}\frac{N - (j - 1)}{j} \bigg/ \prod_{j=1}^{i}\frac{N - (j - 1)}{j}\right\} \\ &= k[ln\,(N - i) - ln\,(i + 1)],\end{aligned} \tag{17}$$

as the increment of entropy between the $(i + 1)$-th and the ith steps of consecutively introducing particles into the system containing N sites. Assuming i to be a continuous variable, one can formally use the Taylor expansion:

$$\Delta S_i = \sum_{k=1}^{\infty}\frac{(\Delta i)^k}{k!}\frac{d^k S(i)}{di^k},$$

so that Eq. 17 reduces to the differential equation

$$\begin{aligned}\frac{dS(i)}{di} &\simeq k[ln\,(N - i) - ln\,(i + 1)] \\ &\simeq k[ln\,(N - i) - ln\,i],\end{aligned} \tag{18}$$

if only the first term is preserved in the expansion. The integral of Eq. 18 has a view:

$$S(i) = k[N\, ln\, N - (N - i)\, ln\, (N - i) - i\, ln\, i], \quad (19)$$

where $S(0)$ is put equal to zero. Substituting $i = n$ into (19) and comparing the result with (16), one can see their coincidence if the approximate Stirling formula: $k! \simeq \exp[k(ln\, k - 1)]$ is applied to the second expression. We may, therefore, conclude that the presence of big ensembles of particles: n, $N - n \gg 1$ within the layer considered is the only condition of validity for the method used. The determination of probabilities, $P_q(j)$, carried out in the same way (see Appendix), gives an additional confirmation of the conclusion obtained.

We now must discuss a model of interactions between the membrane and cytoskeleton in order to finish the problem. However, it is convenient to do this later after the free energy of a separate layer will be determined explicitly.

FURTHER DEVELOPMENT

In the subsequent analysis we will confine ourselves only to the case of the "large persistent length" (i.e., $d_0/\lambda_0 \gg 1$), as is usually said in the polymer physics. The use of such a restriction follows from the fact that microtubules are preferentially oriented parallel to the membrane surface. Besides, the results derived are simpler under this condition than those of the general case of arbitrary values of d_0/λ_0. Additional details have been discussed in the Appendix. As shown there, the free energy of a separate layer of the uniform array considered is given by the expression:

$$\bar{f}_c \simeq 2N\left\{\epsilon_{11}\left(\frac{n}{N}\right)^2 + 2\epsilon_{12}\frac{n}{N}\left(1 - \frac{n}{N}\right) + \epsilon_{22}\left(1 - \frac{n}{N}\right)^2\right\} + u_0 n - kTn\left[1 - \left(\frac{n}{N}\right)^2\right]\langle ln\, m_j\rangle, \quad (20)$$

where

$$\langle ln\, m_j\rangle \simeq ln\, a - \frac{3}{\alpha}\frac{bf(0)}{a}e_1 - \frac{3}{\alpha}\sum_{i=1}^{\infty}\frac{(-1)^i}{i^2}\left[\frac{bf(0)}{a}\right]^i \quad (21)$$

is some mean value averaged over microtubular q-configurations containing vacancies (i.e., $q \neq 0$). The parameters included into Eq. 21 are determined by the relations:

$$a = \frac{3 - n/N}{1 + n/N}; \quad b = 2\Big/\left[1 - \left(\frac{n}{N}\right)^2\right];$$
$$f(0) = \frac{n}{N}\left(1 - \frac{n}{N}\right); \quad \alpha = 3 - \left(1 - \frac{n}{N}\right)^2. \quad (22)$$

It is necessary to add here that the enthalpy terms connected with microtubular bendings are absent in Eq. 20 due to both the supposition made ($d_0/\lambda_0 \gg 1$) and the smallness of bending modules of polymer molecules usually taking place.

As follows from the results derived, the dependence of the entropy term, $kTn[1 - (n/N)^2]\langle ln\, m_j\rangle$, on the composition, n/N, is mainly determined by the number of holes, $n[1 - (n/N)^2]$, which have vacancies (one or two) as the nearest neighbors and, therefore, can undergo bendings. Actually, the number of continuations, m_j, varies from 3 to 1 at the transition from $n/N \Rightarrow 0$ to $n/N \Rightarrow 1$, respectively, so that the resultant change of $\langle ln\, m_j\rangle$ is ~2 times: $1.1 \geq \langle ln\, m_j\rangle \geq 0.48$ (see Appendix). This interval should be somewhat extended in the general case if an arbitrary orientation of microtubular bendings were considered. However, a weak dependence of $\langle ln\, m_j\rangle$ on the composition should also take place under these conditions, since the corresponding changes of $\langle ln\, m_j\rangle$ are negligible compared with those of $n[1 - (n/N)^c]$, where c is the coordination number. This is just the conclusion that allows a simple generalization of the treatment.

The coexistence of phases (for instance, a liquid and its vapor) is usually discussed in the hole theory of liquids followed here. In the Appendix we have touched upon this important point, but the question needs a thorough examination connected with numerical calculations. A complete elucidation of the question seems to be more appropriate in the next communication. Nevertheless, it should be pointed out here that the partition of the system into two phases of different microtubular densities is probably possible at small values of the parameter $w = (\epsilon_{11} + \epsilon_{22} - 2\epsilon_{12}/kT)$ (see Appendix).

We have not so far used in a complete form the initially introduced assumption that microtubular bendings are settled at planes parallel to the membrane. Under the assumption accepted, microtubules can not abandon the layer containing them, and, consequently, the entropy, $\bar{S}^{(i)}$, of the ith layer of a nonuniformed M-layered lattice should only depend on the density n_i/N. This permits the following generalization of Eq. 20:

$$\begin{aligned}\bar{f}_c^{(i)} = {} & N\left\{\epsilon_{11}\left(\frac{n_i}{N}\right)^2 + 2\epsilon_{12}\frac{n_i}{N}\left(1 - \frac{n_i}{N}\right) + \epsilon_{22}\left(1 - \frac{n_i}{N}\right)^2\right\} \\ & + \frac{1}{2}N\left\{\epsilon_{11}\frac{n_i}{N}\frac{(n_{i+1} + n_{i-1})}{N}\right. \\ & + \epsilon_{12}\left[\frac{n_i}{N}\left(2 - \frac{(n_{i+1} + n_{i-1})}{N}\right)\right. \\ & \left. + \left(1 - \frac{n_i}{N}\right)\frac{(n_{i+1} + n_{i-1})}{N}\right] \\ & \left. + \epsilon_{22}\left(1 - \frac{n_i}{N}\right)\left(2 - \frac{(n_{i+1} + n_{i-1})}{N}\right)\right\} \\ & + u_0 n_i - kTn_i\left[1 - \left(\frac{n_i}{N}\right)^2\right]\langle ln\, m_j^{(i)}\rangle \\ & (i = 2, 3, \cdots M), \end{aligned} \quad (23)$$

for the case of a nonhomogeneous square lattice with the forbidden transitions of particles from one layer to another. We will once again emphasize that the quantity $\langle ln\, m_j^{(i)} \rangle$ in this equation is given by Eqs. 21 and 22 with the only difference that index "i" should be attributed to the number of microtubules, n.

At this point, one can include in the consideration performed the interactions between the membrane and cytoskeleton (i.e., the case of $i = 1$). To do this, we suppose like before the inner membrane surface to be divided into the same amount of sites, N, so that a part of these, $m_0/N < 1$, contains specific proteins providing the anchorage of the adjacent cytoskeleton units to the membrane. The remainder of the membrane, $1 - (m_0/N)$, is then to be occupied by some vacancies. Extending indices l, k to the values 3, 4 to denote anchors and vacancies on the membrane, one can treat quantities ϵ_{lk} as the energies per length d_0 of all the possible pairwise interactions between particles l and k. The subsequent introduction of the membrane-cytoskeleton interaction into the free energy of the system is carried out in the following way. The factors $(n_2 + n_0)/N$ and $(2 - [(n_2 + n_0)/N])$ appearing in Eq. 23 at $i = 1$ are replaced with $(n_2 + m_0)/N$ and $[(1 - (n_2/N)) + (1 - (m_0/N))]$, and the energies ϵ_{13}, ϵ_{14}, ϵ_{23}, ϵ_{24} are attributed to the generated products $n_1 m_0/N$, $(n_1/N)(1 - (m_0/N))$, $(1 - (n_1/N))(m_0/N)$, $(1 - (n_1/N))(1 - (m_0/N))$, respectively, so that the resultant expression has the form:

$$\begin{aligned}\bar{f}_c^{(1)} = {} & N\left\{\Delta\epsilon\left(\frac{n_1}{N}\right)^2 + 2(\epsilon_{12} - \epsilon_{22})\frac{n_1}{N} + \epsilon_{22}\right\} \\ & + \frac{N}{2}\left\{\Delta\epsilon\,\frac{n_1 n_2}{N^2} + (\epsilon_{12} - \epsilon_{22})\frac{(n_1 + n_2)}{N} + \epsilon_{22}\right\} \\ & + \frac{N}{2}\left\{\left[\epsilon_{13}\frac{m_0}{N} + \epsilon_{14}\left(1 - \frac{m_0}{N}\right)\right]\frac{n_1}{N}\right. \\ & \left. + \left[\epsilon_{23}\frac{m_0}{N} + \epsilon_{24}\left(1 - \frac{m_0}{N}\right)\right]\left(1 - \frac{n_1}{N}\right)\right\} + u_0 n_1 \\ & - kTn_1\left[1 - \left(\frac{n_1}{N}\right)^2\right]\langle ln\, m_j^{(1)}\rangle. \qquad (24)\end{aligned}$$

Because the total number of layers, M, is assumed to be large (that means, in particular, the absence of the membrane–membrane interaction through the cytoskeleton arranged between them), there is no necessity to specify the model further by considering the free energy of the membrane itself. It is only required to increase twice the terms $N/2\{[\epsilon_{13}(m_0/N) + \epsilon_{14}(1 - (m_0/N))](n_1/N) + [\epsilon_{23}(m_0/N) + \epsilon_{24}(1 - (m_0/N))](1 - (n_1/N))\}$ included into Eq. 24 to take into account completely the membrane-cytoskeleton interactions.

Summarizing the relations derived over i from $i = 1$ to $i = M$, we get the free energy of the system as the given function of the variables n_i/N and the parameters ϵ_{lk}, u_0, T: $\bar{F}_c = F(n_i/N, u_0, \epsilon_{lk}, T)$. The anchor density, m_0/N, might be also considered as a parameter of the system under the conditions discussed. However, in a general case (for instance, two membrane system with the limited intermembrane distance, M), this quantity will become an additional variable.

We are now in a position to perform a minimization of $\bar{F}_c$ to find out the equilibrium distribution of the cytoskeleton density, n_i/N, as a function of number i (or the distance from the membrane). To do this, one must consider independent variations in quantities n_i limited by the condition of the constancy of cytoskeleton units: $\sum_{i=1}^{M} n_i = \text{Const}$. This gives the set of equations:

$$\left(\frac{\partial \bar{F}_c}{\partial n_i}\right)_{n_j,N} = \mu \quad (i \neq j,\ i = 1, 2, \ldots, M), \qquad (25)$$

where μ is the Lagrange factor to be determined from the above condition. From the equilibrium conditions given by Eqs. 25, it follows that densities $x_i = n_i/N$ should satisfy the equation:

$$\begin{aligned} & x_{i+1} - 2x_i + x_{i-1} - \frac{kT}{\Delta\epsilon}\left(\frac{\partial}{\partial x_i}[x_i(1 - x_i^2)\langle ln\, m_j^{(i)}\rangle]\right)_{N,T} \\ & \qquad - 2(1 - 2x_i) = \frac{1}{\Delta\epsilon}[2(\epsilon_{22} - \epsilon_{11}) - u_0 + \mu], \\ & \qquad \Delta\epsilon = \epsilon_{11} + \epsilon_{22} - 2\epsilon_{12} \qquad (26)\end{aligned}$$

for all values of i except for the case of $i = 1$. The equation:

$$\begin{aligned} & x_2 - 2x_1 - \frac{kT}{\Delta\epsilon}\left(\frac{\partial}{\partial x_1}[x_1(1 - x_1^2)\langle ln\, m_j^{(1)}\rangle]\right)_{N,T} \\ & \quad - 2(1 - 2x_1) = \frac{1}{\Delta\epsilon}\left[4(\epsilon_{22} - \epsilon_{12}) - 2\Delta\epsilon - u_0 + \mu + \epsilon_{12}\right. \\ & \quad \left. + \epsilon_{24} - \epsilon_{14} - \epsilon_{22} - \frac{(\epsilon_{13} + \epsilon_{24} - \epsilon_{23} - \epsilon_{14})}{N} m_0\right], \qquad (27)\end{aligned}$$

playing the role of the boundary condition to the set of Eqs. 26, is valid for this case. However, the latter can be included into the previous set of Eqs. 26 if we define the quantity x_0 to be equal to $(1/\Delta\epsilon)[\epsilon_{14} + \epsilon_{22} - \epsilon_{12} - \epsilon_{24} - (\epsilon_{14} + \epsilon_{23} - \epsilon_{13} - \epsilon_{24})(m_0/N)]$, having used the constancy of the density m_0/N assumed earlier. The introduced parameter x_0 thus is dependent on both the density m_0/N and the relative affinities $\epsilon_{lk}/\Delta\epsilon$. Therefore, it might accept values not belonging to the interval (0.1) which is its principal difference as compared with other x_i.

It can be shown that the Lagrange factor μ coincides with the chemical potential of cytoskeleton units (for the definition of this property see Appendix). Consequently, the latter preserves its value throughout all the layers of the volume considered according to Eqs. 25 (or Eqs. 26). The equations derived are similar to the so-called Ono equations of an interfacial layer (Ono and Kondo, 1960) and can be solved by means of an appropriate numerical method. Here, we will use another method that seems to be more informative than numerical calculations.

As it was mentioned above there exist different approaches in the theory of surface properties of liquids. In the scope of the continuous approach (Cahn and Hilliard, 1958), it is assumed "that the composition gradient is small compared with the reciprocal of the intermolecular distance," so that the free energy $\bar{f}$ per particle might be considered as a continuous function of the distance from the interface. This is equivalent to the point representation of particles, and, hence, the total free energy of a nonuniform system is to be determined by

$$\bar{F} = AN_{\mathrm{v}} \int_{(v)} \bar{f}\, dz,$$

where N_{v} is the number of particles per unit of the volume V of the system; A, the cross-section area, and z, the distance from the interface. The authors supposed $\bar{f}$ to be "the sum of two contributions which are functions of the local composition, $x(z)$, and the local composition derivatives," $x^{(\mathrm{k})}(z)$. As a result, there appears a variational problem of finding the distribution, $x(z)$, of particles which would give an extremum of the total free energy, $\bar{F}$. Because the free energy must be invariant to the symmetry operation of reflection ($z \Rightarrow -z$) for an isotropic (but nonhomogeneous) medium, the following expansion of $\bar{f}$ can be used for the case of a flat interface:

$$\bar{f} = \bar{f}_0(x(z)) + k_1 \frac{d^2x(z)}{dz^2} + k_2\left(\frac{dx(z)}{dz}\right)^2 + \cdots$$

Applying this expansion and neglecting terms in derivatives higher than the second, Cahn and Hilliard derived the Euler equation of the variational problem. As with Ono's equation, the derived one also implies the constancy of the chemical potential throughout the system. Therefore, it is quite natural to think that transition to the description discussed can be performed at the level of Ono's equations (or Eqs. 26 in our case). This is exactly the way we are using here without bothering to substantiate the details. However, a few words should be said.

The ideal multilayered lattice used previously is a certain approximation to reality, especially given that microtubules might have local bendings within the segments considered. As a result, some distortions of the lattice should inevitably appear. Supposing such distortions to be nonregular and slowly oscillated from one segment of the system to another, one can apply the expansion

$$x_{\mathrm{i}}(S_{\mathrm{i}} + \Delta) = x_{\mathrm{i}}(S) = \sum_{k=0}^{\infty} \frac{\Delta^{\mathrm{k}}}{k!} \frac{d^{\mathrm{k}}x_{\mathrm{i}}(S_{\mathrm{i}})}{dS^{\mathrm{k}}} = \sum_{k=0}^{\infty} \frac{1}{k!} \frac{d^{\mathrm{k}}x_{\mathrm{i}}(z_{\mathrm{i}})}{dZ^{\mathrm{k}}}, \quad (28)$$

to describe changes in density x_{i} of the ith layer which occur under variations Δ in the distances $S_{\mathrm{i}} = i\lambda_0$ (or $Z_{\mathrm{i}} = S_{\mathrm{i}}/\lambda_0 = i$) corresponding to the ideal lattice. Moreover, Eq. 28 might be used for the case of $|\Delta| = \lambda_0$, and, thereby, the second difference, $x_{\mathrm{i}+1} - 2x_{\mathrm{i}} + x_{\mathrm{i}-1}$, contained into Eqs. 26 can be replaced with the second derivative

$$x_{\mathrm{i}+1} - 2x_{\mathrm{i}} + x_{\mathrm{i}-1} \simeq d^2x(Z_{\mathrm{i}})/dZ^2$$

with the accuracy to the fourth order terms that corresponds to Cahn and Hilliard's supposition about the smallness of the composition gradient. Thus, the difference equations of the lattice approach reduce to the differential form:

$$\frac{d^2x(Z)}{dZ^2} = \frac{kT}{\Delta\epsilon}\left(\frac{\partial}{\partial x}[x(1 - x^2)\langle ln\ m_{\mathrm{j}}(x)\rangle]\right)_{\mathrm{N,T}} + 2(1 - 2x) + \frac{1}{\Delta\epsilon}[2(\epsilon_{22} - \epsilon_{11}) - u_0 + \mu], \quad (29)$$

where index i is omitted since the result obtained is valid for any Z by assumption.

The above provides only a hint of the validity of the transition performed. But its correctness is clear since Eq. 29 coincides with the one which can be derived from strict considerations, *mutatis mutandi* in the Cahn and Hilliard procedure.

The first integral of Eq. 29 is

$$\left(\frac{\mathrm{d}x}{\mathrm{d}Z}\right)^2 = -4(x - x_\infty)^2 + \frac{2kT}{\Delta\epsilon} x(1 - x^2)\langle ln\ m_{\mathrm{j}}(x)\rangle\big|_{x_\infty}^{x} - \frac{2kT}{\Delta\epsilon}(x - x_\infty) \cdot \left(\frac{\partial}{\partial x_\infty}[x_\infty(1 - x_\infty^2)\langle ln\ m_{\mathrm{j}}(x_\infty)\rangle]\right)_{\mathrm{N,T}}, \quad (30)$$

where the condition: $\lim_{z\to\infty} x(Z) = x_\infty$ (or $dx/dZ \Rightarrow 0$ at $Z \Rightarrow \infty$; x_∞, the bulk density of cytoskeleton) has been taken into account.

The analysis of the equation derived, and its difference predecessor (Eq. 26) as well, is not trivial and needs significant increase in the volume of the paper. Numerous and important consequences of such analysis will be reported in the next paper. Here we only point out the following results. Solutions of these equations correspond to a minimum of the free energy $\bar{F}_{\mathrm{c}}$ only at values of parameter $\Delta\epsilon = \epsilon_{11} + \epsilon_{22} - 2\epsilon_{12}$, satisfying the inequality

$$\Delta\epsilon > -\tfrac{1}{2}kT, \quad (31)$$

as it follows from the sufficient condition of the positivity of the second differential, $d^2\bar{F}_{\mathrm{c}}$ (or a corresponding quadratic form in the case of the lattice treatment). This means that only the case of positive and small negative values of $\Delta\epsilon$ can be considered in the scope of the model discussed. However, as it will be explained in the next paragraph, this is just the case that is most interesting for practical applications. Within the region of $\Delta\epsilon$ given by the above condition, the distribution of the cytoskeleton density along distance Z resembles an exponential one the exponent of which depends on the value of $\Delta\epsilon$ in an extreme manner. In particular, maximum values of the exponent take place when parameter $\Delta\epsilon$, which can be conventionally called the lattice energy, is much smaller than the heat: $\Delta\epsilon/kT \ll 1$. If the positive lattice energy is comparable or larger than the heat, the dependence discussed is insignificant, but it occurs to be a strong one for admissible negative values of $\Delta\epsilon$. Speaking in lattice terms of description, the submembrane cytoskeleton (i.e., the region of variable cytoskeleton densities) should include no more than two to three layers of the lattice at $\Delta\epsilon > kT$, but it becomes practically infinite when the lattice energy accepts a critical value $\Delta\epsilon = -\frac{1}{2}kT$. Only one-layered submembrane cytoskeleton is possible at zero value of $\Delta\epsilon$. Taking these results into account and recalling the initial estimate of the width of submembrane cytoskeleton ($\sim 10\lambda_0$), one can primarily conclude that the case of negative lattice energies comparable with $\frac{1}{2}kT$ is most favorable for the discussion of nerve fiber cytoskeleton occurring in reality.

DISCUSSION

Postponing the general discussion of the approach proposed to the next paper, we would like, nevertheless, to consider here some points which seem to be important for further developments. First consider the initial supposition about practically infinite length of microtubules. A more distinctive formulation of such a supposition may be possible, and that will permit us to extract additional consequences. To do this, recall the previously indicated analogy between the configuration of a bending microtubule and the path described by a diffusing particle.

Having proved theoretically the appearance of submembrane cytoskeleton in the model discussed, we have the possibility to estimate correlation radius L_x for the latitudinal and normal (to the membrane) directions. As has been pointed out earlier, the width of submembrane cytoskeleton and, thereby, correlation radius L_x is somewhere about twice the lattice constant ($2\lambda_0$) at $\Delta\epsilon \geq kT$ and significantly increases with approaching the lattice energy to the critical value equal to $-\frac{1}{2}kT$. Having the estimates of L_x, we can now calculate correlation radius L_l in the longitudinal direction by using the analogy mentioned above. According to diffusion theory, the mean-square displacements, $\langle Z^2 \rangle$, of a diffusing particle (a microtubule axis, in our case) are given by the Einstein-Smoluchovsky relation:

$$\langle Z^2 \rangle = D\Delta t, \tag{32}$$

where D, the diffusion coefficient; Δt, the time of observation. "Inasmuch as the particle moves with a fixed average velocity at a given temperature, it is evident that the time (Δt) corresponds to the chain length of the polymer" (Flory, 1953), that is, to the length L of a microtubule from an origin. Obviously, a free path between consecutive collisions of the particle is equal to the persistent length d_0 in our terms. As a result of a single "collision", a microtubule axis can undergo the single displacement equal to λ_0 provided that the microtubule chosen belongs to the subgroup $\{m\}$ of bending microtubules from the total set, $n\{1 - (n/N)^2\}$, of particles which are able to bend. This gives, in particular,

$$D = \left\{ \frac{m\lambda_0}{n\left[1 - \left(\frac{n}{N}\right)^2\right]} \right\}^2 \cdot \frac{1}{d_0} \approx 0.3\,\frac{\lambda_0^2}{d_0}, \tag{33}$$

where the averaged value of $\{(m/n[1 - (n/N)^2])\}^2$ is accepted. Thus, Eq. 32 reduces to

$$\langle \Delta Z^2 \rangle \approx 0.3\,\frac{\lambda_0^2}{d_0}\,L \tag{34}$$

under the definitions stated above. Now, it is evident that correlation radius L_l should correspond to the distance satisfying the condition

$$L_l \approx \frac{d_0}{0.3\lambda_0^2}\,L_x^2, \tag{35}$$

because the achievement of the mean displacement $\langle \Delta Z \rangle \approx \langle \Delta Z^2 \rangle^{1/2}$ equal to L_x is the natural definition of the quantity qualitatively introduced earlier. As one can also see from Eq. 35, there was no necessity to assume the persistent length d_0 to be large to parallelize microtubules to the membrane. Even if $d_0 \simeq \lambda_0$ and $L_x \simeq 2$ the ratio L_l/L_x is ~ 10, which provides the required parallel orientation. It is obvious that Eq. 35, being a necessary consequence of the analogy between the two phenomena compared, should be by order valid for arbitrary values of coordination number $c > 2$. Therefore the relation obtained seems to be useful to analyze macrofluctuations in the cytoskeleton density occurring in reality, as clearly seen from Fig. 1. There are also other possibilities to consider inhomogeneities of cytoskeleton in the scope of the approach developed. They result from the principal opportunity to use other distributions of quasiparticles in the initial state than those given by Eqs. 6, 6′. We would like to add here that the existence of periodic oscillations of the composition (within the range of a separate layer, at least) follows from an evident generalization of Eq. 29 on the two-dimensional case. A corresponding differential equation must obviously include the second partial derivative of the density with respect to the distance, y, along the latitudinal direction (see Cahn and Hilliard, 1958). However, the possibilities indicated need a more detailed consideration.

The last thing to be done at this point is to estimate how large the length of a microtubule, L_0, must be in order that the approach used would be valid. The latter will certainly occur if the length L_0 exceeds correlation radius L_l:

$$L_0 \geq 10 L_L \approx 30\left(\frac{L_x}{\lambda_0}\right)^2 d_0 \qquad (36)$$

that gives the estimate: $L_0 \geq 10^2\lambda_0$ at $L_x \simeq 2\lambda_0$ and $d_0 = \lambda_0$. Thus, the length of quasiparticles L_0, being equal to or greater than 20 μm, should be assumed, provided that $\lambda_0 \approx 0.2$ μm, as was initially estimated (see Introduction). Such values of L_0 seem to be acceptable, but the length required must be larger for larger correlation radius L_x, as seen from Eq. 36. This means, that some generalization of the theory on the case of finite length L_0 probably needs to describe nerve fiber cytoskeleton with values $L_x \simeq 10\lambda_0$. A suitable extension of the theory seems possible and, moreover, is now in preparation. However, we can certainly affirm here that the conclusions to be obtained will not undergo significant changes compared with the derived ones. This certitude is due to our previous investigation (Malev and Gromov, 1990) where an alternative model of cytoskeleton has been analyzed. In that paper, we have, in a sense, postulated the expression for the free energy of a single layer $f_c^{(i)}$ which differs from the derived one (Eq. 23) only in the entropy $\bar{S}^{(i)}$. The latter has been put equal to the configuration entropy of a layer

$$\bar{S}^{(i)} = -(N - n_i)\, ln\left(1 - \frac{n_i}{N}\right) - n_i\, ln\frac{n_i}{N},$$

(compare with Eq. 19 at $i = n_i$), which is, as a matter of fact, equivalent to the coincidence of L_0 with the segment length d_0. Thus, the results of the above paper correspond to the second limiting case of a small length of the particles forming cytoskeleton. We will carry out an appropriate comparison between the results of both cases later, but their qualitative resemblance might be pointed out here. It is clear that the existence of submembrane cytoskeleton should follow from both models because only quantitative changes accompany the transition from one case to another. Therefore, one can think that the molecular theory of capillarity used here provides, at least, a qualitative description of cytoskeleton structures. Because the possibility of varying parameters of the model applied also exists, there is the chance to achieve a quantitative agreement between theoretical and experimental results. In this connection, we indicate that a lattice energy $\Delta\epsilon$ approximately equal to $-kT$ must be assumed to obtain theoretically the dependence given by Eq. 1 (Malev and Gromov, 1990). As has been indicated earlier, practically the same requirement presumably follows from the above consideration so that an independent justification of such a conclusion is needed.

One can see from Fig. 4, that the parameter λ_0 of the lattices formed by microtubules within thin axons of *Carcinus maenas* leg is close to three times the diameter of a microtubule: $\lambda_0 \approx 0.1$ μm which is by order in agreement with the previous estimate: $\lambda_0 \approx 0.2$ μm from Sakai's results. This implies that interactions of a site occupied by a microtubule with nearest neighbors (i.e., energies ϵ_{1k}, $k = 1, 2$) should be mainly determined by water–water interaction ϵ_{22} so that the lattice energy $\Delta\epsilon = \epsilon_{11} + \epsilon_{22} - 2\epsilon_{12}$ occurs to be zero or of a small value compared with its constituents. This assertion is more correct the lower the probability of finding cross-bridges between microtubules. Besides, such bridges observed in reality (Sakai et al., 1985) force out some amount of water molecules from the site volume, and, hence, the resulting effect might be negligible. Apart from the reasons indicated above, small values of $\Delta\epsilon$ seem to be true because the axon's perfusion easily destroys the cytoskeleton even if such a process is not long (Terakawa, 1985). This means a smallness of the lattice energy and, hence, is in accord with the conclusion obtained. One more circumstance may be looked upon as an additional confirmation of the above conclusion. Under small lattice energies comparable with the heat, thermal fluctuations should affect the structure of cytoskeleton. As a result, macroscopic inhomogeneities in the cytoskeleton density at a given distance from a cellular membrane must appear. Because such inhomogeneities are actually observed in reality (see Fig. 1), it is safe to say that the theory right reflects the peculiarities of nerve fiber cytoskeleton. This once again confirms the initial assumption that the cytoskeleton organization in nerve fibers results from the same physical reasons which are responsible for the existence of interfacial layers of more simple systems.

Certainly, there are other questions to be discussed. This will be done later, but good possibilities of the approach proposed are clearly seen at the present moment.

APPENDIX

Free energy of a uniform lattice

The contents of this part of the paper is based on the results published recently in Russian (Malev, 1991), and we believe that it is worthwhile to repeat them here for the convience of Anglo-American reading audience.

The following remark is useful before the analysis to be performed. Microtubules, whose q-configuration does not contain vacancies (i.e.,

$q = 0$), might be continued into the next segment only with a single (nonbending) way. Therefore, one can exclude such quasi-particles from the product $\bar{W}$ by changing the definition of the latter as

$$\bar{W} = \prod_{j=1}^{n_1} m_j \qquad (A.1)$$

where $n_1 = n \sum_{q=1}^{2} C_2^{(q)}(n/N)^{2-q}(1 - n/N)^q = n[1 - (n/N)^2]$, the total number of microtubules having vacancies (one or two) as the nearest neighbors.

Set the quantity $j_q(j)$ to be the amount of microtubules of q-configuration within the l-th segment which were continued into the $(l + 1)$th one by the moment j of the continuation process. Then, the difference, $nC_2^{(q)}(n/N)^{2-q}(1 - n/N)^q - j_q(j)$, gives the amount of noncontinued particles of the same q-configuration in the lth segment, so that the probability of finding such microtubules $P_q(j)$ at the jth step is

$$P_q(j) = \left[nC_2^{(q)}\left(\frac{n}{N}\right)^{2-q}\left(1 - \frac{n}{N}\right)^q - j_q(j)\right] \Big/ (n_1 - j), \qquad (A.2)$$

where $n_1 - j$, the total number of noncontinued particles within the lth segment at the same moment. On the other hand, $P_q(j)$ is obviously equal to the ratio $\Delta j_q / \Delta j$ where Δj_q is the number of the particles considered which have been continued for the interval Δj of the process. Using the expansion

$$\Delta j_q = \sum_{k=1}^{\infty} \frac{(\Delta j)^k}{k!} \frac{d^k j_q(j)}{dj^k}, \qquad (A.3)$$

where only the first term (with $k = 1$) is retained, we get the equation:

$$dj_q/dj = \left[nC_2^{(q)}\left(\frac{n}{N}\right)^{2-q}\left(1 - \frac{n}{N}\right)^q - j_q(j)\right] \Big/ (n_1 - j),$$

which gives the following results:

$$j_q = \frac{n}{n_1} C_2^{(q)}\left(1 - \frac{n}{N}\right)^q\left(\frac{n}{N}\right)^{2-q} j \quad \text{and}$$

$$P_q = \frac{n}{n_1} C_2^{(q)}\left(1 - \frac{n}{N}\right)^q\left(\frac{n}{N}\right)^{2-q}, \qquad (A.4)$$

under the evident condition $j_q = 0$ at $j = 0$. The relations derived would be trivial if there were not the previous exclusion of non-bending particles from the continuation procedure.

Thus, neglecting possible deviations of the probabilities P_q from their mean values given by Eq. A.4, the numeric sequences $\{j\}$, $\{j_q(j)\}$ can be formally considered as continuous ones. In a certain extent, one can take into consideration such deviations by preserving the second term, $\tfrac{1}{2}(\Delta j)^2 d^2 j_q / dj^2$, of Eq. A.3 and assuming Δj equal to 1. Recalling the previous illustration of the transition proposed (see Theory section), one must regard it to be justified if numbers of particles of any q-configuration are sufficiently large.

The same method is applied to seek the relative number of blocked vacancies in the $(l + 1)$-th segment, $\lambda(j)$, by the moment j. To do this, write the differential equation

$$d[1 - \lambda(j)]/dj = -\frac{1}{(N - n)_2} dj_\lambda(j)/dj, \qquad (A.5)$$

that is just the definition (in the differential form) of $\lambda(j)$ provided that $j_\lambda(j)$ is the number of bending continuations into the vacancies, being the blocking ones within the lth segment, up to the moment j, and $(N - n)_2 = (N - n)(n/N)^2$, the total number of blocking holes in the lth segment. The derivative $dj_\lambda(j)/dj$, having the meaning of the probability of the bending continuation of a microtubule in a blocking hole, is nothing else than the sum over pq-configurations:

$$dj_\lambda(j)/dj = \sum_{q=1}^{2} P_q \sum_{p=0}^{q} P_q^{(p)} \sum_{i=0}^{q-p} C_{q-p}^{(i)}[\lambda(j)]^i[1 - \lambda(j)]^{q-p-i} \cdot (q - p - i)/(q - i + 1) \qquad (A.6)$$

of the products of the probabilities of finding a microtubule in pq-configuration with i blocked vacancies, $P_q P_q^{(p)} C_{q-p}^{(i)}[\lambda(j)]^i \cdot [1 - \lambda(j)]^{q-p-i}$, times on the probability, $(q - p - i)/(q - i + 1)$, of a bending continuation of the microtubule into a blocking hole being yet nonblocked up to the moment j. Substituting Eq. A.6 into Eq. A.5, we get the differential one

$$df/dj = \frac{\beta}{\alpha} f(f - \alpha), \qquad (A.7)$$

where $\alpha = 3 - (1 - (n/N))^2$, $\beta = \alpha/3n_1$ and $f(j) = (n/N)(1 - (n/N))[1 - \lambda(j)]$. From Eq. (A.7) it follows,

$$f(j) = \alpha f(0) e_j / [\alpha - f(0)(1 - e_j)], \qquad (A.8)$$

with parameters $f(0)$ and e_j defined by

$$f(0) = \frac{n}{N}\left(1 - \frac{n}{N}\right); \quad e_j = e^{-\beta j} = \exp\left(-\frac{\alpha j}{3n_1}\right). \qquad (A.9)$$

The same method is applied to determine the total number of microtubules, m, suffering bending continuations into blocking and nonblocking holes as well. Omitting details of such a derivation, the following result is obtained:

$$\begin{aligned} m = n\left(1 - \frac{n}{N}\right)\Bigg\{&\frac{\alpha}{3}\left(1 - \frac{n}{N}\right) + \left(1 - \frac{n}{N}\right) \\ &\times \ln\left[1 - \frac{f(0)}{\alpha}(1 - e_1)\right] + \frac{n}{N}(1 - e_1) \\ &\cdot \left[1 - \frac{f(0)}{\alpha}\right] \Big/ \left[1 - \frac{f(0)}{\alpha}(1 - e_1)\right]\Bigg\}, \\ e_1 = e^{-\alpha/3}&. \end{aligned} \qquad (A.10)$$

It remains only to point out here that the number of continuations of the jth microtubule is given by

$$\begin{aligned} m_j = \frac{n}{n_1} \sum_{q=1}^{2} C_2^{(q)}&\left(\frac{n}{N}\right)^{2-q}\left(1 - \frac{n}{N}\right)^q \\ &\times \left[1 + \sum_{p=0}^{q} pP_q^{(p)} + \frac{f(j)}{\frac{n}{N}\left(1 - \frac{n}{N}\right)} \cdot \sum_{p=0}^{q} (q - p)P_q^{(p)}\right], \end{aligned} \qquad (A.11)$$

where Eq. 9 have not been used for the sake of brevity. Using Eqs. A.8 and A.11, we are able to calculate the entropy, $\bar{s}$, of an organization of the subsequent segment of the system from the preceding one. This is performed in the same method that was repeatedly used above. Namely, considering the entropy increment, $\Delta\bar{s}(j) = k \ln [\bar{W}(j + 1)/\bar{W}(j)] \cong k \ln m_j$, we can put it equal to the derivative, $d\bar{s}(j)/dj$, so that the entropy will be given by

$$\bar{s}(j) = k \int_0^j \ln m_j \, \mathrm{dj} = \int_{f(0)}^{f(j)} (\ln m_j)\left(\frac{df}{dj}\right)^{-1} df \qquad (A.12)$$

where $\ln m_j$ and $f(j)$ are determined by Eqs. A.11 and A.8, respectively.

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Because all the quantities required are known, one can write the following results for the enthalpy

$$\bar{h} = 2N\left\{\epsilon_{11}\left(\frac{n}{N}\right)^2 + 2\epsilon_{12}\frac{n}{N}\left(1-\frac{n}{N}\right) + \epsilon_{22}\left(1-\frac{n}{N}\right)^2\right\}$$
$$+ u_0 n + (\lambda_0 u_0/d_0 + 2\mu_\beta)n\left(1-\frac{n}{N}\right)$$
$$\times\left\{\frac{\alpha}{3}\left(1-\frac{n}{N}\right) + \left(1-\frac{n}{N}\right)\ln\left[1-\frac{f(0)}{\alpha}(1-e_1)\right]\right.$$
$$\left. + \frac{n}{N}(1-e_1)\frac{1-f(0)/\alpha}{1-\dfrac{f(0)}{\alpha}(1-e_1)}\right\}, \qquad (A.13)$$

and the entropy constituents of the free energy

$$T\bar{s} = \frac{3kT}{\alpha}n\left[1-\left(\frac{n}{N}\right)^2\right]\left\{\ln(a+b\alpha)\cdot\ln\left[\frac{f(n_1)-\alpha}{f(0)-\alpha}\right]\right.$$
$$- \ln a\cdot\ln\frac{f(n_1)}{f(0)} + \sum_{i=1}^{\infty}\frac{(-1)^i}{i^2}\left[\left\{\left[\frac{f(n_1)}{f(0)}\right]^i - 1\right\}\right.$$
$$\left.\left.\times\left\{\frac{bf(0)}{a}\right\}^i + \left\{\frac{b[f(0)-\alpha]}{(a+b\alpha)}\right\}^i\left\{1-\left[\frac{f(n_1)-\alpha}{f(0)-\alpha}\right]^i\right\}\right]\right\}$$
$$(A.14)$$

where $a = (3-(n/N))/(1+(n/N))$, $b = 2/[1-((n/N))^2]$, and $f(n_1)$ follows from Eq. A.8 at $j = n_1$. Applying the law of the mean to Eq. A.12,

$$\bar{s} = \bar{s}(n_1) = k\int_0^{n_1}\ln m_j\,dj$$
$$= k\langle\ln m_j\rangle\int_0^{n_1}dj = kn\left[1-\left(\frac{n}{N}\right)^2\right]\langle\ln m_j\rangle$$

and comparing the result obtained with Eq. A.14, we find

$$\langle\ln m_j\rangle = \frac{3}{\alpha}\left\{\ln(a+b\alpha)\cdot\ln\left[\frac{f(n_1)-\alpha}{f(0)-\alpha}\right] - \ln a\cdot\ln\frac{f(n_1)}{f(0)}\right.$$
$$+ \sum_{i=1}^{\infty}\frac{(-1)^i}{i^2}\cdot\left[\left\{\frac{bf(0)}{a}\right\}^i\left\{\left[\frac{f(n_1)}{f(0)}\right]^i - 1\right\}\right.$$
$$\left.\left.+\left\{\frac{b[f(0)-\alpha]}{(a+b\alpha)}\right\}^i\left\{1-\left[\frac{f(n_1)-\alpha}{f(0)-\alpha}\right]^i\right\}\right]\right\} \qquad (A.15)$$

The mean quantity $\langle\ln m_j\rangle$ is to be considered as a slowly varying function of the composition, n/N, compared with the total amount of microtubules, $n[1-(n/N)^2]$, which can undergo bendings. Actually, as seen from Eq. A.15, values of $\langle\ln m_j\rangle$ lie in the interval (1.1, 0.48) whose upper and lower bounds correspond to $n/N \Rightarrow 0$ and $n/N \Rightarrow 1$, respectively. Therefore, the entropy changes with the composition are practically determined only by the factor $n[1-(n/N)^2]$ in the model discussed. It is obvious, that this conclusion will be also valid for any reasonable coordination number c because possible changes in m_j are negligible compared with the composition ones. The results obtained for the case of $c = 4$ will be published later.

Some simplifications of the results derived are possible if the smallness of $f(0)/\alpha = (n/N)(1-(n/N))/[3-(1-(n/N))^2] < (n/2N) \times (1-(n/N)) < 1/8 \ll 1$ is taken into account. In particular, the expressions:

$$\bar{f} \simeq 2N\left\{\epsilon_{11}\left(\frac{n}{N}\right)^2 + 2\epsilon_{12}\frac{n}{N}\left(1-\frac{n}{N}\right) + \epsilon_{22}\left(1-\frac{n}{N}\right)^2\right\}$$
$$+ u_0 n + \left(2\mu_b + \frac{\lambda_0 u_0}{d_0}\right)\cdot n\left(1-\frac{n}{N}\right)\left\{1-\frac{n}{N}e_1\right.$$
$$\left. -\frac{1}{3}\left(1-\frac{n}{N}\right)^3 - \frac{f(0)}{\alpha}(1-e_1)\left[1-\frac{n}{N}(1-e_1)\right]\right\}$$
$$- kTn\left[1-\left(\frac{n}{N}\right)^2\right]\langle\ln m_j\rangle \qquad (A.16)$$

and

$$\langle\ln m_j\rangle \simeq \ln a - \frac{3bf(0)}{\alpha a}e_1 - \frac{3}{\alpha}\sum_{i=1}^{\infty}\frac{(-1)^i}{i^2}\left[\frac{bf(0)}{a}\right]^i \qquad (A.17)$$

are valid with the accuracy to the second order of $f(0)/\alpha$.

The osmotic pressure, Π, and the chemical potential of cytoskeleton quasi-particles, μ, are to be found from Eqs. (A.16, 17) to describe completely the uniform lattice (i.e., volume cytoskeleton considered). The first one is determined by the partial derivative $(\partial\bar{f}/\partial N)_{T,n_s} = -\Pi$ (More exactly to say, that $(\partial\bar{f}_c/\partial N)_{T,n}$ determines a two-dimensional osmotic pressure, because the segment length d_0 remains constant at variations of N in a cross-section of a layer.), where $n_s = n + m\lambda_0/d_0$ is the total content of cytoskeleton substances within the segment chosen, and the derivative $(\partial\bar{f}/\partial n_s)_{T,N} = \mu$ gives the second quantity required. As has been pointed out above, microtubules seem mainly to be parallel to the membrane. Therefore, it is reasonable to suppose that the ratio λ_0/d_0 is small, that is the so-called case of large persistent length d_0, occurs place under the experimental conditions. In this case, we get

$$\Pi = -\left(\frac{\partial\bar{f}}{\partial N}\right)_{T,n_s} \simeq -\left(\frac{\partial\bar{f}}{\partial N}\right)_{T,n}$$
$$\simeq -2\epsilon_{22} + 2\Delta\epsilon\left(\frac{n}{N}\right)^2 + 2kT\left(\frac{n}{N}\right)^3\langle\ln m_j\rangle$$
$$+ kTn\left[1-\left(\frac{n}{N}\right)^2\right]\left(\frac{\partial}{\partial N}\langle\ln m_j\rangle\right)_{T,n},$$
$$\Delta\epsilon = \epsilon_{11} + \epsilon_{22} - 2\epsilon_{12}, \qquad (A.18)$$

and

$$\mu = \left(\frac{\partial\bar{f}}{\partial n_s}\right)_{T,N} \simeq \left(\frac{\partial\bar{f}}{\partial n}\right)_{T,N} \simeq u_0 + 4(\epsilon_{12}-\epsilon_{22}) + 4\Delta\epsilon\frac{n}{N}$$
$$- kT\left\{\left[1-3\left(\frac{n}{N}\right)^2\right]\langle\ln m_j\rangle\right.$$
$$\left. + n\left[1-\left(\frac{n}{N}\right)^2\right]\left(\frac{\partial}{\partial n}\langle\ln m_j\rangle\right)_{T,N}\right\}, \qquad (A.19)$$

where terms proportional to the bending energy μ_β are omitted due to its smallness.

It has been established previously that $\langle\ln m_j\rangle$ is a slowly varying function of the composition so that a simple representation: $\langle\ln m_j\rangle = \epsilon_0 - \epsilon_1(n/N) - \epsilon_2(n/N)^2$, $\epsilon_0 = 1.1$; $\epsilon_1 + \epsilon_2 = 0.62$; $\epsilon_1, \epsilon_2 > 0$, or something like it, can be used to calculate analytically the derivatives included in Eqs. A.18, A.19. This gives, for instance, the relation:

$$\Pi + 2\epsilon_{22} = \left(\frac{n}{N}\right)^2 \left\{ 2\Delta\epsilon + kT\epsilon_1 + 2kT(\epsilon_0 + \epsilon_2)\frac{n}{N} - 3kT\epsilon_1\left(\frac{n}{N}\right)^2 - 4kT\epsilon_2\left(\frac{n}{N}\right)^3 \right\}, \quad (A.20)$$

which shows the possibility of the van der Waals character of the isotherm $\Pi(n/N)$ under small negative values of $\Delta\epsilon > -kT$. In other words, there might exist some region of the isotherm where pressure Π, not being a single-valued function of n/N, has the same magnitude for three different densities. This creates premises for the partition of the system into two phases of different compositions, similar to that which takes place for pure liquids under temperatures smaller than the critical temperature T_c (Ono and Kondo, 1960). But something more than the estimates made above is required to prove that it will be the case for the system considered here. To be brief, we indicate only that numerical calculations are probably inevitable during the analysis to be performed. In the forthcoming paper there will be also an extension of the method applied above to the case of a nonhomogeneous lattice where the entropy of a single layer depends in explicit manner on the densities of adjacent layers.

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