CELL BIOPHYSICS 5, 95-104 (1983)

On Spatial Periodicity in the Formation of Cell Adhesions to a Substrate

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Received January 15, 1983; Accepted March 22, 1983

Abstract

Interference reflection microscopy reveals a fairly regular array of marginal focal contacts in early states of cell spreading. A theoretical explanation of the onset of patchiness is given, based on the positively cooperative binding to the substrate of laterally diffusing receptor molecules.

Index Entries: Cell spreading; adhesion, of cells, periodic, model of; focal contacts, of cells; positive cooperativity, of cells forming adhesions; reaction–diffusion, of cells forming adhesions; periodicity, in the formation of cell adhesions; spreading, of cells forming adhesions.

Introduction

Studies on the adhesion and spreading of cultured cells using electron microscopy or interference reflection optics point to a considerable complexity in organization of contact regions at the ventral cell surfaces [Harris, (*1*); Trinkaus (*2*); Vasiliev

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0163-4992/83/0600-0095$02.00

and Gelfand (*3*); Grinnell (*4*); Heaysman and Pegrum (*5*)]. This heterogeneity could arise from a number of factors, both extrinsic and intrinsic, including nonuniform organization of the extra-cellular matrix, chemotactic stimuli, contacts with neighboring cells, as well as intracellular constraints. Using electron microscopy [Abercrombie et al. (*6*)] immunofloresence microscopy, and interference reflection optics [Curtis (*7*); Izzard and Lochner (*8, 9*); Geiger (*10*)] or a combination of these [Heath and Dunn (*11*); Wehland (*12*)] two major classes of adhesion sites were defined. Closest and probably strongest attachments, called *focal contacts* or *focal adhesion plaques* (apparent distance between cell and substrate 100–150 Å) occurred in restricted areas over the ventral surfaces of the cells. These regions produce interference–reflection dark images as shown in Fig. 1. *Close contacts* are somewhat less firm attachments (300 Å distance between cell and substrate) that produce gray interference–reflection images [Izzard and Lochner (*8, 9*)].

In the early stages of adhesion when the cells undergo "radial spreading" [Vasiliev and Gelfand (*3*); Grinnell (*4*)] a regular periodic arrangement of radial focal contacts may be observed near the cell periphery. This pattern of cell–substrate contact is accompanied or followed by intracellular reorganization of microfilaments and associated proteins—notably vinculin, α-actinin, and actin—into nearly overlapping membrane-associated patches [Geiger (*10, 13, 14*)].

The progress of adhesion during early stages of cell spreading, as visualized by interference reflection microscopy, is shown in Fig. 1. Initially, the ventral cell membrane forms a nearly uniform belt of close contact at the periphery, as in Fig. 1A. Subsequently (within 15–30 min after plating) small radial patches of nascent focal contact can be detected, as marked in Fig. 1B. In later stages (30–60 min) the entire periphery of the cell contains radial periodic focal contacts (Fig. 1C). When the cell starts to move and acquires polar shape the periodicity is lost and the typical pattern of contact sites is obtained [Fig. 1D; see Izzard and Lochner (*8, 9*); Heath and Dunn (*11*); Geiger (*10*); Heaysman and Pegrum (*5*) for comparison].

Any theory of cell contact and spreading must explain the observed periodic structures. The object of this paper is to take a first step in the formulation of such a theory.

Although the periodic focal contacts are seen by interference reflection optics and immunolabeling for vinculin, we suggest that the periodicity may already be manifested at an earlier stage of contact, when cell surface molecules M make their first binding with substrate molecules S, to form a cell–substrate complex C. It has already been hypothesized that in later stages such complexes initiate bindings with intracellular vinculin, which in turn anchors bundles of microfilaments to the contact region [Geiger (*13, 14*)]. This process may modify and stabilize the periodic structures formed by a spatially nonuniform distribution of complexes.

We will construct a mathematical model with the aid of which we can demonstrate the possibility of initiating the formation of the observed spatially periodic structures. Moreover, the model will show how alterations of certain parameters are expected to affect the wavelength of the periodicity. In principle, at least, this opens the door to an experimental challenge of the theory.

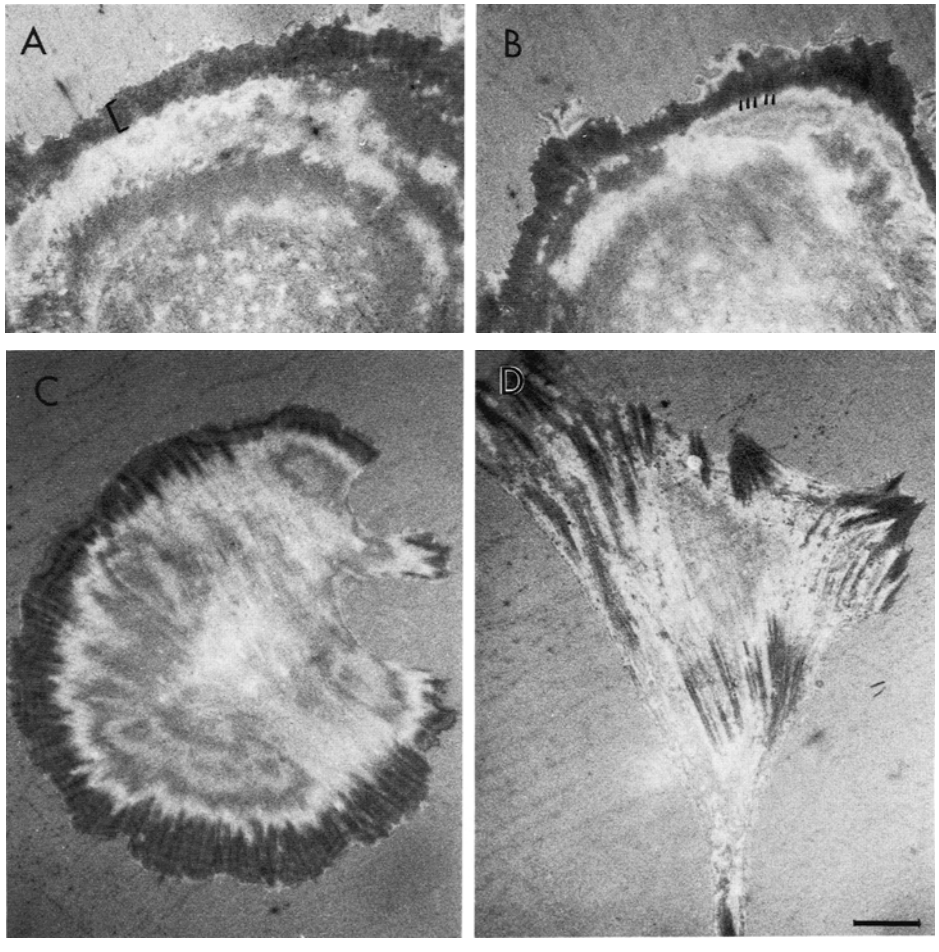

Fig. 1. Interference reflection microscopy of living chicken gizzard cells at different intervals after plating. A and B: Two adjacent cells 15 min after plating. C. 30 min after plating. D. 3 h after plating. Notice the nearly uniform close contact in the early stages, represented by the peripheral belt (marked with bracket). Nascent radial focal contacts can be detected in A and B (small patches are indicated by the bars in B) and become prominent in C.

The Model

The relevant cell-contact activities take place near the periphery of the cell. We will assume that the curvature of this region is of secondary importance, so that we can "straighten out" the initial contact region and describe position along the boundary by a linear spatial variable x. Our aim, then, is to predict the development of complex concentrations C that are periodic in x.

We assume that both the surface molecules M and the complexes C are laterally mobile with diffusion coefficients D_M and D_C, respectively. (The same letters M and C are used for the names of the molecules and for their concentrations.) Diffu-

sion coefficients such as D_M for protein molecules embedded in membranes have been measured by various means [see Schlessinger and Elson (*15*)] and typically are of magnitude 10^{-9} cm^2/s. In connection with receptor diffusivity, we call attention to the review by Bell (*16*) of work by him and others on how diffusivity affects contact by influencing such factors as receptor collision rates and the accumulation of receptors in an initial contact area.

The quantity D_C is an effective diffusion constant in the following sense. We envision the receptor molecule to be in two main states, in proximity to the membrane (C) or not (M). When in proximity, the receptor is envisioned to remain more or less firmly bound to a site on the membrane for a time of τ seconds and then to "hop" randomly to a nearby site, which is an average distance of δ cm away. By random walk calculations of the type described for example in Lin and Segel (*17*), Section 3.3, such behavior can be described as diffusion with an effective diffusion constant of $\delta^2/4\tau$ cm^2/s.

Our key assumption is that the formation at some point of a cell–substrate complex C makes it more likely that another such complex will be formed in the neighborhood of that point—and makes it less likely that already formed complexes will break apart (see Fig. 2). If we symbolize the binding kinetics by

$$M + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C \qquad [1]$$

then our assumption can be expressed by postulating that the "on" rate constant k_1 at point x is an increasing function of $C(x)$ while the "off" rate constant k_{-1} is a decreasing function of $C(x)$ (see Fig. 3). In effect, we are postulating a positive cooperativity in membrane–substrate binding.

From Eq. [1], the rate at which complexes C are formed is k_1SM. We will assume that the sites S at which binding can take place are so large in number that S can be regarded as constant. To simplify the notation a little, we can then introduce the effective first-order forward rate constant $k_+ = k_1S$. With this, the equations for the concentrations $M(x, t)$ and $C(x, t)$ as functions of position x and time t can be written

$$\partial M/\partial t = -k_+M + k_{-1}C + D_M\partial^2 M/\partial x^2 \qquad [2a]$$

$$\partial C/\partial t = k_+M - k_{-1}C + D_C\partial^2 M/\partial x^2 \qquad [2b]$$

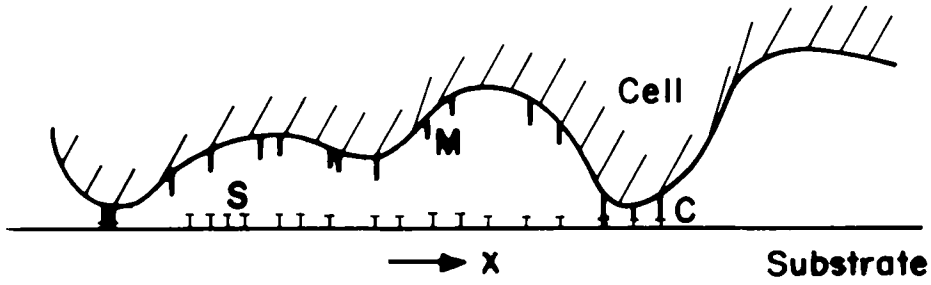

Fig. 2. Cell surface molecules M bind with substrate S to form complex C. Locally, formation of complex C restricts membrane motion and enhances further net binding.

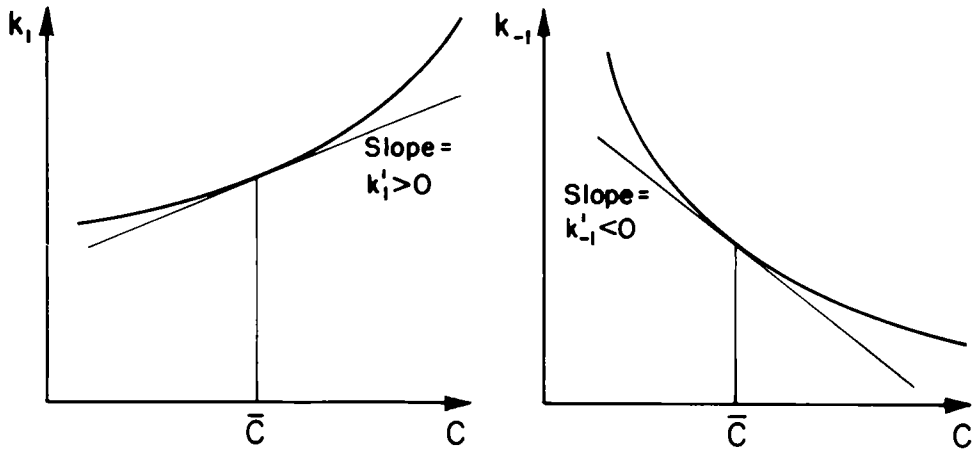

Fig. 3. Dependence of "on" rate constant k_1 and "off" rate constant k_{-1} on complex concentration C. Near steady state $C = \bar{C}$ the graphs can be approximated by straight lines of slopes k_1' and k'_{-1}, respectively.

We also assume that possible net production of M receptors may be ignored during the initial stages of contact formation (for a period of seconds to minutes) so that the average number of receptors, both free and bound, can be regarded as constant. We denote this by M_0.

Our problem now is to demonstrate that the Eqs. [2] possess a spatially periodic solution that is compatible with the observations. A direct demonstration would require the overcoming of formidable mathematical difficulties, so that we proceed indirectly (and conventionally) as follows. We first show that the governing Eqs. [2] possess a uniform solution, corresponding to a permanent distribution of unbound and bound receptors that does not depend on the spatial variable x. We then examine solutions that are initially "almost" uniform. For certain parameter ranges we find that such solutions rapidly tend to become more and more uniform. The uniform solution is thus stable to the small disturbances that inevitably occur in a real system, and for these parameter ranges we consequently would expect to observe an essentially uniform situation. For other parameter values, however, we demonstrate that small disturbances to uniformity will start to grow. Now the model predicts that the observed state of the system cannot be a uniform one. Under such circumstances, we can conjecture that a "patchy" distribution of receptors will result. Furthermore our relative simple analysis allows us to make some tentative predictions concerning the size of the patches.

The Uniform State and Its Stability

The governing Eqs. [2] possess a spatially uniform time-invariant solution wherein $M = \bar{M}$, $C = \bar{C}$, $\bar{M}$ and $\bar{C}$ constants. From Eq. [2]

$$-k_1\bar{M} + k_{-1}\bar{C} = 0 \qquad [3a]$$

Since $\bar{M} + \bar{C} = M_0$, we find that

$$\bar{C} = M_0/(1 + K_d) \qquad [3b]$$

where

$$K_d \equiv k_{-1}(\bar{C})/k_+(\bar{C}) \qquad [4]$$

is the dissociation constant when $C = \bar{C}$. (See Appendix for further discussion.) In our attempt to find spatial inhomogeneity, we now examine the stability of the uniform solution to small disturbances. To this end, we introduce perturbations $M'(x, t)$ and $C'(x, t)$ from the steady state, where

$$M'(x, t) = M(x, t) - \bar{M},\ C'(x, t) = C(x, t) - \bar{C} \qquad [5]$$

Upon substituting $M = M' + \bar{M}$, $C = C' + \bar{C}$ into Eq. [2] we obtain equations in the perturbation variables M' and C'. In a standard type of analysis (see Appendix) we convert the perturbation equations into an easily soluble form by discarding all (relatively small) nonlinear terms. It turns out that it is sufficient to examine solutions with the special form

$$M'(x, t) = \hat{M}e^{\sigma t} \cos \alpha x,\ C'(x, t) = \hat{C}e^{\sigma t} \cos \alpha x \qquad [6]$$

where $\hat{M}$, $\hat{C}$, σ, and α are constants. These solutions are spatially periodic with wave number α. That is, there are α waves per unit length, and the spatial period L is given by $L = 2\pi/\alpha$.

Calculations outlined in the Appendix provide us with a value of the *growth rate* $\sigma(L)$ for each wave length L. If $\sigma(L) < 0$, then perturbations of the wavelength in question will decay exponentially in time. But if $\sigma(L) > 0$, then the theory predicts exponential growth of a perturbation of wavelength L. In the present case, growing disturbances are found if and only if a certain quantity W is positive, where W is the following combination of parameters:

$$W = \frac{M_0}{1 + K_d}\left(\frac{-k'_{-1}}{k_+} + K_d\frac{k'_+}{k_+}\right) - \left(K_d + \frac{D_C}{D_M}\right) \qquad [7]$$

Appearing in W are the quantities k'_{-1} and $k'_+ \equiv k'_1 S$, where k'_{-1} and k'_1 are the slopes defined in Fig. 3.

Let us consider this result with some care. If $W < 0$, then $\sigma(L) < 0$ for all L. Small sinusoidal disturbances to uniformity (of the form given in Eq. [6]) will therefore decay, whatever their wavelength L. The theory of Fourier analysis shows that all reasonable small disturbances can be formed by summing terms of the type considered in Eq. [6]. Thus *all* small disturbances to uniformity will decay if $W < 0$—which in particular will occur if the positive quantities $-k'_{-1}$ and k'_+ are small enough. In other words, if there is only a small amount of "positive cooperativity" in binding, then the uniform state is *stable* to small disturbances.

If the uniform state is stable, then we expect to observe such a state. Since we observe *non*uniformity, within our theoretical framework we are driven to assume that the instability condition $W > 0$ holds. In such a case, disturbances of wavelength L are predicted by our theory to grow exponentially [$\sigma(L) > 0$] when

$$\alpha^2 \leq k_+ W/D_M \qquad [8a]$$

i.e., when

$$L \geq 2\pi[D_M/(k_+W)]^{1/2} \qquad [8b]$$

We have thus demonstrated the existence of conditions under which the uniform state cannot be expected to appear, for inevitable small disturbances are bound to grow. A major qualitative feature of the observations, spatial nonuniformity, can thus be explained. But there are two defects in the theory as it stands: (i) continual exponential growth of the perturbations is predicted and (ii) there is no prediction to compare with the observed wavelength of the spatial nonuniformity.

In answer to objection (i) we note that however small the perturbations were initially, if $W > 0$ then the growth of these perturbations will eventually make them "large." When this occurs the linearization of the equations for the perturbations M' and C' no longer provides a good approximation, and our results cease to be valid. That is, our linearized analysis can describe the initial growth of perturbations but cannot tell their eventual fate. Experience of many authors with problems of this kind [see for example Segel and Levin (*18*)] suggests that the perturbations will eventually cease to grow, so that the concentrations $M(x, t)$ and $C(x, t)$ will probably approach a new spatially periodic stationary state of some wavelength L_{SS} (Fig. 4).

This brings us to objection (ii). Experience also indicates that the spatial wavelength L_{SS} of the new steady state will be given, *roughly*, by the wavelength of the growing set [8b] whose growth rate σ is maximum. If W is only slightly positive (uniform state "barely" unstable) then the maximum can be shown [as in Segel (*19*)] to occur for the wavenumber α_{SS} that is in the middle of the permitted range of α^2 given by Eq. [8a]:

$$\alpha_{SS}^2 = k_+W/(2D_M) \qquad [9a]$$

so that

$$L_{SS} = 2\pi/\alpha_{SS} = 2\pi[2D_M/(k_+W)]^{1/2} \qquad [9b]$$

Even in more general conditions of instability we expect that Eq. [9b] will provide a reasonable estimate for the observed wavelength.

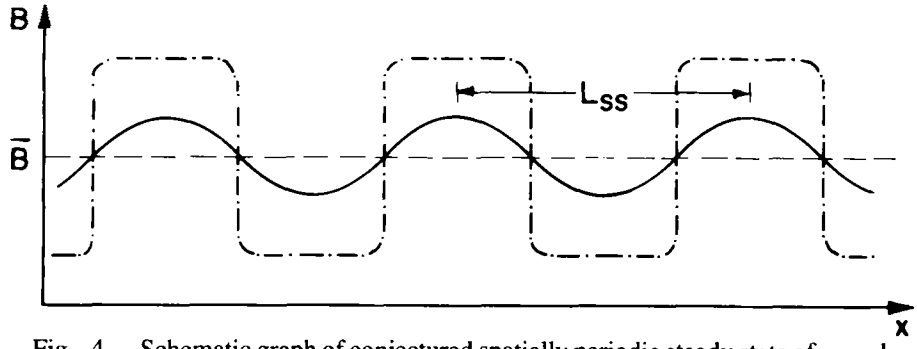

Fig. 4. Schematic graph of conjectured spatially periodic steady state of complex concentration when $W > 0$. A rough estimate for the wavelength L_{SS} is given in Eq. [9]. Two possible types of steady state are shown, almost sinusoidal (—) and patchy (— . —). The latter is expected when D_C is small or zero, by analogy with the ecological study of Murray and Mimura (*22*).

Discussion

We have presented observations that indicate that the onset of cell adhesion exhibits a spatially periodic or "patchy" structure at the cell periphery. Such observations provide a macroscopic consequence of the molecular events that constitute the early stages in cell adhesion, and they thereby provide a benchmark for any proposed molecular model of the principal adhesion events. We have presented such a model, wherein the first events are the binding of laterally diffusing surface molecules to the substrate. By analyzing "small" disturbances (less than about 10% of the uniform concentrations $\bar{M}$ and $\bar{C}$), we have shown that spatial inhomogeneity will automatically appear if the binding and diffusivity parameters are such that the parameter combination W is positive, where W is defined in Eq. [7]. The clustering is promoted by an assumed "positive cooperativity" wherein the formation of one cell-substrate bond at a point makes it more likely that other bonds will appear in the neighborhood.

The patchiness that occurs if $W > 0$ is an example of a reaction–diffusion instability of the type that has been vigorously investigated in developmental biology and ecology [as reviewed for example in Segel (*20*), Section 6.4]. These instabilities require short-range activation and long range inhibition. Here the activation comes through the positive cooperativity associated with the complex C, and indeed Eq. [7] shows that the patchiness will not occur if the ratio D_C/D_M is too large. [Segel and Jackson (*21*) provide a more detailed explanation for such instabilities.]

The influence of the diffusion coefficients on the predicted patch period L_{SS} can be found from Eqs. [9b] and [7]. In principle at least, this affords an opportunity for testing the theory. If one increases the effective complex diffusivity D_C by using a less adhesive substrate (as long as the increase is not so great as to render W negative), then there should be a tendency toward larger periods. Addition of agents to the membrane could modify the diffusion coefficient D_M, but this provides a less effective challenge to the theory, since an increase in patch period can be brought about either by an increase or a decrease in D_M, depending on the other parameters.

It might be asked why the observed adhesion plaques are elongated and not roundish. Within the confines of the model, a choice of these alternatives can be made by a somewhat complicated calculation that takes into account the nonlinear terms [see for example Segel and Levin (*18*)]. Such a calculation does not seem justified in view of the tentative nature of the model. Moreover the explanation may be connected with factors that are legitimately neglected in a first model. For example, it is possible that contact spots that are initiated as described here are later stabilized by elongated filamentous structures (actin bundles) that effect the shape of the growing contact regions. In addition, overall movement of peripheral lamellipodia during spreading or locomotion may effect the growth direction of the focal contact.

In conclusion, we suggest that it would be worthwhile to attempt to find agents that will alter the observed spatial periodicity, or to find and characterize mutants with different spatial periods. This suggestion flows from the fact, demonstrated

here, that the presence of spatial periodicity and the wavelength of the period may provide important clues to the nature of the molecular mechanisms that bring about cellular adhesion.

Appendix: Some Theoretical Details

For a general background discussion of the linear stability theory that we employ, the reader is referred to Segel (*20*), Section 6.5. There a problem somewhat analogous to the present one is considered; conditions are derived for the clumping of identical chemotactic organisms by examining the stability of a uniform state of cells and attractant.

We remark that Eq. [3b] can be regarded as providing the steady state value $\bar{C}$ as the intersection of the straight line $y = C$ with the graph of $y = M_0/[1+K_d(C)]$. We assume circumstances such that there will be a unique intersection.

Given Eq. [6], the linearized problem reduces to

$$\begin{pmatrix} -(k_+ + D_M\alpha^2) & Q \\ k_+ & -(Q + D_C\alpha^2) \end{pmatrix} \begin{pmatrix} \hat{M} \\ \hat{C} \end{pmatrix} = \sigma \begin{pmatrix} M \\ \hat{C} \end{pmatrix} \qquad [A1]$$

where

$$Q \equiv k_{-1} - k'_+ \bar{M} + k'_{-1}\bar{C}$$

Standard theory shows that instability can occur via oscillatory growth (σ complex) if the trace of the $\times$ 2 matrix in [A1] is positive, i.e ., if

$$\alpha^2 < -(k_+ + Q)/(D_M + D_C) \qquad [A2]$$

Monotonic growth occurs if the determinant is negative, i.e., if (cf. Eq. [8])

$$\alpha^2 < -(k_+D_C + D_MQ)/D_C \qquad [A3]$$

If α^2 is to be positive [A2] requires $Q < -k_1$ while [A3] requires

$$Q < -(D_C/D_M)k_1$$

Since we expect that $D_C < D_M$ (probably $D_C << D_M$) the latter condition is less restrictive; we thus anticipate monotonic instability.

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