$Y_1, Y_2, \ldots$ be a sequence of independent two-valued random variables, $Y_{n+1} = -s$ or $\beta + ns$ with probabilities $(1 - W)$ and $W$, where $s$ is a small positive number and $W$ is then determined by the condition $-E(Y_n) = \alpha V(Y_n)$. Verify that the probability that for some $n$, $-\beta + Y_1 + \ldots + Y_n \geq 0$ converges to $1/(1 + \alpha \beta)$ as $s \to 0$, and let $X_n = Y_n - E(Y_n)$. This completes the proof.

The theorem can be extended to say that for each $\gamma > 0$, if $\tau_\gamma$ is the least $n$ if any for which $(X_1 + \ldots + X_n) \leq -\gamma + (\mu_1 + \ldots + \mu_n) + \alpha(V_1 + \ldots + V_n)$, then the probability that there is some $n < \tau_\gamma$ for which (1) holds is less than $(\gamma/(\gamma + \beta))(1/(1 + \alpha \beta))$; and this bound is sharp.

The material of this note, including proofs of Lemmas 1 and 2, will appear as part of our forthcoming book, *How to Gamble If You Must* (New York: McGraw-Hill), Theorems 2.12.1 and 9.4.1, and an illustrative application of the theorem will appear in the forthcoming article, "A sharper form of the Borel-Cantelli lemma and the strong law" by L. E. Dubins and D. A. Freedman.

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**CYTOPLASMIC INHERITANCE OF THE ORGANIZATION OF THE CELL CORTEX IN PARAMECIUM AURELIA**

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The cortex of *Paramecium aurelia* exhibits a high degree of structural differentiation (Fig. 1). At each level of organization observable within the limits of resolution of the optical microscope, the cortical pattern is remarkably constant and reproduces faithfully through a regular cycle of changes during growth and fissions. However, this highly stable organization can be experimentally modified. Sonneborn obtained several variants of the normal pattern by fusion of mates after conjugation, by partial loss of supernumerary structures, and by "cortical picking," an accident of separation after conjugation in which one of the mates acquires a piece of its partner. Sonneborn showed, by all available methods of genetic analysis, that these abnormal patterns behaved like "cortical mutations." Each variation proved to be hereditary although the variants were genotypically identical to wild type, as though the existing pattern of cortical organization itself determined the pattern that arose during subsequent reproduction. This ordering of new by old cell structures, which has been called "macrocrystallinity" or "cytotaxis," might be an important, although still little recognized, mechanism in cell heredity. We therefore undertook further experiments and extended the analysis to more elementary levels of structural organization.

The analysis exploited various grafts of a piece of one cell on a whole cell, using stock 51 (syngen 4) of *P. aurelia*. All of these arose from conjugating pairs which remained united by a cytoplasmic bridge instead of separating immediately after conjugation. Some pairs eventually separated spontaneously in such a way that a
part of one conjugant went to the other. In other pairs most of one conjugant was
cut off with a micromanipulator, the residual part persisting as a graft on the other.

The progeny of animals bearing such spontaneous and experimentally produced
grafts were observed from fission to fission in order to follow the development of the
grafted piece. The methods of culture and handling of the organism have been
described elsewhere.4 For detailed study of the cortical geography, specimens were
prepared by a modification of the silver nitrate impregnation technique6 and pieces
of isolated cortex by a simplification of the digitonin technique. Each method
revealed details of organization not shown by the other.

Determination at the Level of the Repeating Unit of Cortical Structure.—Origin and
nature of the mutant “twisty”: This mutant was derived from an exconjugant that
received a piece of its mate during spontaneous breakage of the conjugation bridge.
The piece formed a little tail near the posterior end on the ventral side. Because of
its posterior position, it passed to successive opisthes (posterior products of trans-
verse fission), but became less and less obvious, disappearing by the fourth fission.
Nevertheless, the clone derived from the fourth successive opisthe displayed ab-
normal swimming, designated as “twisty.” This gives the impression of marked
twisting during progression forward, but is merely an exaggeration of normal spiral
progression.

Twisty swimming has a structural basis which appears from a comparison of
normal (Fig. 1) with twisty (Fig. 2) cells. Unlike the regular spacing of the rows of
cortical granules in normal cells (Fig. 1A,B), cells of the twisty clone (Fig. 2) show
a patch of four rows bounded on one side by an unusually wide space and, on the
other side, by an unusually narrow space. The intervening rows consist of cortical
units with reversed polarities: instead of the normal polarity described in the
Figure 1, legend the kinetosomes lie to the left (instead of the right) of the unit mid-
line, the parasomal sacs are to the left (instead of the right) of the kinetosomes, and
the kinetodesmal fibers (see Figs. 1C and 3) emerge to the left (instead of the right)
of the kinetosomes and extend posteriorly (instead of anteriorly). This reversal of
both antero-posterior and right-left polarities will be symbolized henceforth by
RP; it is equivalent to 180° rotation in the plane of the body surface. Such rows of
RP units constitute the first reported exception to the rule of desmodexy,7 the
rule that kinetodesmal fibers in Ciliates always lie to the right of the kinetosomes.
This exception explains the abnormally wide and narrow spaces at the edges of the
patch of RP rows in the twisty clone (Fig. 2, rpr). When a row of normal units has
a row of RP units on its left, the larger clear areas of units in both rows are adjacent
and the space therefore appears unusually wide. Conversely, when an RP row has
a normal row on its left, the visible structures of both rows are brought into juxta-
position, leaving hardly any clear space between them.

Experimental production of cells with rows of RP cortical units: The simplest
hypothesis as to the origin of the twisty clone is that the tail of the ancestral excon-
jugant included a piece of cortex of its mate which became assimilated in reversed
orientation. This assumption is reasonable, for exconjugant pairs held together
by a cytoplasmic bridge often twist into a settled heteropolar position (Fig. 4A).
Moreover, it can be verified experimentally. By cutting off most of one cell from
a heteropolar pair of exconjugants, one can observe that the remaining fragment
becomes grafted to the intact cell and that the progeny include twisty sublines of
descent. But the easiest way to obtain clones of cells with complete rows of RP cortical units was the following.

Pairs of conjugants united in heteropolar position (Fig. 4A) were isolated. Such pairs grow and divide and at each fission (Fig. 4B, C, D) give off a proter from each end while, in the central heteropolar double opisthe, the zone of union extends progressively. By about the third fission (Fig. 4D) the opisthes are fused up to or beyond the vestibular region, so that the fission furrows cut through a mixed cortex. At this stage each newly arising protor has most of its cortex derived from its "own" parent cell, but contains a little heteropolar bit in its posterior end. If such a cell is cultured, the normal process of cortical growth associated with fission brings about, in successive opisthes, the extension forward of the "grafted" rows. In the third opisthe they extend all the way to the anterior pole. All the clones derived from isolation of the third opisthe of third protors given off by a heteropolar pair displayed the twisty swimming behavior. Each clone was characterized by a distinctive number (up to 12) of rows of RP cortical units inserted as a single patch or as two or three patches separated by a few normal rows.  

Inheritance of rows of RP cortical units: The original twisty, as well as the many twisty clones obtained by the procedure just described, reproduced true to type through hundreds of fissions, dozens of autogamies, and conjugations with normals, with or without endoplasmic exchanges. Occasionally one or more RP rows could be lost from a particular subline of descent.

This persistence of rows of RP cortical units can be understood from Dippell's\(^8\) analysis of production of new cortical units. She has shown that new structures (kinetosomes, parasomal sacs, and kinetodesmal fibers) arise within each "old" unit in locations and with polarities that are fixed in relation to the "old" components of the unit. For example, new kinetodesmal fibers grow out from kinetosomes on the same side and in the same direction as the old ones, and comparable definite spatial relationships are shown by new and old kinetosomes and parasomal sacs. Then, longitudinal growth of cortical membranes and appearance of transverse partitions yield identically organized units from each old one. In this way the units of a row are traceable to a common ancestral unit, the orientation of which is always perpetuated by the same mechanism, regardless of the orientation of the unit and of the polarity of the cell.

Determination at the Level of Differentiated Fields of Cortical Rows.—The question may now be asked: what determines the path followed by a row of cortical units and (or) what determines the differentiation of groups of rows into characteristic patterns (Fig. 1A, B): circumoral and vestibular fields around the ingestory apparatus, right fields of rows running from anterior suture to posterior pole, left fields running from anterior to posterior sutures, and dorsal field extending from pole to pole?

First, as became evident from further observation of the twisty clones, the paths followed by the rows are \textit{not} intrinsic properties of any of them. All RP rows were initially located close to the suture line on the right side. In the course of hundreds of fissions, they were observed to be located progressively further to the right, eventually reaching the left side of the cell. The mechanism of this shift relative to the oral meridian is not yet understood, but it is clearly not peculiar to RP rows, for the normal rows interspersed among them accompany them. As such groups
of normal and RP rows are followed into different fields, they fit the path characteristic of the region in which they come to lie. These paths are therefore somehow imposed on the rows by their location or their "cortical environment." This will now be demonstrated by a second type of observation.

In a pair of exconjugants united by a cytoplasmic bridge, one exconjugant was cut (Fig. 5A, B) so as to remove roughly the anterior 2/3 of the cell including the whole ingestatory apparatus (vestibule, mouth, and gullet). What remains of the posterior left field (Fig. 5B, l2) of the amputated cell (represented in black) is fused to the posterior right field (r1) of the host. Conversely, 180° away (on the side not visible in the figure), what remains of the posterior right field of the amputated cell is fused to the host's posterior left field. The host's vestibule lies anterior to the graft and is surrounded on both sides by only host circumoral material. Figure 5C, D, E shows the development of the graft during the first three fissions. In the first two successive opisthes (A1, B), the cortical growth accompanying fission enlarged the graft forward to the vestibular region. Then by the third fission (Fig. 5E) the "daughter" vestibule, which goes to the opisthe, came to lie at the juncture of the right field (r1) of the host with the left field (l2) of the graft. At the other juncture (180° away) between host and graft cortex, the whole ingestatory apparatus being absent, no circumoral or vestibular fields developed; but at both junctures the other features (anterior and posterior sutures and cytopyge, see Fig. 1B) of the

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Fig. 1.—Normal cortical geography of Paramecium aurelia. (A) Dorsal view, and (B) ventral view, of a silver nitrate impregnated cell. Magnification: × 416. A cell's right and left sides and anterior and posterior ends are defined in relation to the major landmarks of the ventral aspect. "To the right" means clockwise around the body from the anterior (as) and posterior (ps) sutures in (B) when facing the anterior end (top of fig.); the right and left sides [r and l in (A) and (B)] are to the animal's right and left of the sutures, respectively. (C) Diagrammatic enlargement of the unit of cortical structure, including one or two kinetosomes (k) or ciliary basal bodies, slightly to the right of the unit midline, one parasomal sac (psc) to the right of the kinetosome and anterior to it (or to the posterior kinetosome in units possessing two), and one kinetodermal fiber (kf) emerging to the right and extending anteriorly (a) from the kinetosome (or from the posterior one when two are present). The 1 or 2 kinetosomes and parasomal sac of one unit, lying close together, appear as a single granule (silver deposit) in photographs (A) and (B), but they are partially resolvable (see Fig. 2). Kinetodesmal fibers show only in digitonin preparations (see Fig. 3).

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a, anterior. as, anterior suture. c, cytopyge or cell anus. crp, pores of the contractile vacuoles. k, kinetosome. kf, kinetodermal fiber. l, left. p, posterior. ps, posterior suture. psc, parasomal sac. r, right. s, vestibule leading inward to mouth and gullet.

Fig. 2.—Part of dorsal surface of a silver impregnated typical cell of the twisty clone after about 300 cell generations. Magnification: × 1800. In the four rows with reversed polarities (rpr), the compound granules "point" to the observer's right, while in the normal rows (nr) they point to the observer's left (i.e., animal's right).

Fig. 3.—Digitonin preparation of a cell from a clone possessing a single row with reversed polarities (rpr). The kinetodesmal fibers in this row, unlike those in the normal rows (nr), emerge to the animal's left (l) and extend posteriorly (toward bottom of figure). Magnification: × 1800.

Fig. 4.—Origin of cells possessing some rows of cortical units with reversed polarities. (A) Pair of exconjugants united in heteropolar position; (B), (C), and (D), first, second, and third fissions of the heteropolar doublet cell. pr1 and pr1', pr2 and pr2', pr3 and pr3', are the three pairs of proters (anterior fission products) given off from the two anterior ends (a) of the heteropolar doublet at its first three fissions. Small oval are the vestibules leading inward to mouth and gullet. In the diagrams ([A], (B), and (D)), one row of cortical units on either side of the juncture between the two fused cells is represented by a row of triangles pointing in opposite directions. This shows the opposite polarity of the units, the points of the triangles representing the position of the parasomal sacs (see Figs. 1C and 2). The photograph (C) shows that all rows beyond the juncture are oriented like the row nearest the juncture. Note that at the third fission (D), the proters (pr3 and pr3') receive a mixed cortex including oppositely oriented rows on both sides of the juncture line.

Fig. 5.—Origin of clone of incomplete doublets (lacking one ingestatory apparatus) by excision of part of one conjugant of a homopolar fused pair. For description, see text.
midventral meridian were present. Between the two midventral meridians, on both sides, was a "back" with its two contractile vacuole pores (as in Fig. 1A). This incompletely double organization (lacking one ingestory apparatus), which constitutes another type of cortical variation and has been previously described, characterized the clone descended from the third posterior fission product (Fig. 5, o3) of the grafted cell.

The significant features of this development are (1) that the piece of grafted left field (l2) has acquired the vestibular and circumoral differentiations when it became associated with the host's ingestory apparatus, and (2) that the left field of the host, originally possessing the circumoral and vestibular patterns, lost these differentiations when it developed 180° away in the absence of an ingestory apparatus, along the right field of the graft.

In more general terms, the presence or absence of a given cortical organization (ingestory apparatus in this case) determines whether other characteristic patterns (vestibular and circumoral fields in this case) will differentiate during growth of adjacent or nearby regions. This confirms the conclusion already drawn from the behavior of rows of RP cortical units: the cortical environment determines the path or form the rows take. Comparable observations, not reported here, on the differentiation of the cytopyge and the ingestory apparatus show that changes in the surrounding cortical organization result in production of abnormal structures or in failure to form them altogether. In agreement, definite cortical requirements for oral differentiation have also been reported for Stentor⁹ and other Ciliates.

Discussion.—The major conclusion from our results is that, at each studied level, the organization, i.e., presence, orientation, and location, of newly formed cortical structures is determined by the cortical environment existing at the time of their development. This raises the question of the mechanisms of perpetuation of both normal and variant cortical organizations and of the role of genes in this perpetuation.

The variations reported here are perpetuated during sexual and asexual reproduction in the direct cortical line of descent. As set forth previously, the variations persist in the absence of any other known differences between "mutant" and normal cells. The absence of genic and nuclear differences has been demonstrated by the genic and nuclear identities of host and graft and by breeding experiments. The absence of endoplasmic differences has been shown again by endoplasmic identity of host cell and donor cell and, in some cases, by exchange of endoplasm between normal and variants during conjugation. This, of course, does not mean that genes do not play any role in the cortical organization. They are, in all probability, necessary for production of the specific molecules that make the structures, and gene mutations are known which result in altered cortical organization. Nevertheless, the coexistence, on the same cell, of a normal oral meridian and of a deficient one lacking circumoral differentiations, and of normal and RP rows of cortical units, could hardly be accounted for by any noncortical difference, even by differences in local concentrations of genic products since the RP rows, for instance, persist as such as they "move" around the cell.

The mechanism of perpetuation of cortical variations is less reliable than genic mechanisms. Irreversible losses of parts and eventually of all of the variant pattern may occur with a higher order of frequency than mutations or losses of a spe-
specific gene. Nevertheless, certain variants, such as complete doublets and cells with rows of RP cortical units, can apparently be maintained indefinitely with periodic selection. Even those which revert 100 per cent to normal and do so rapidly (for example, incomplete doublets that revert after 30–40 cell generations) transmit the variant organization to a remarkably large number of progeny cells (10^4 to 10^12 in the example cited). Hence, even imperfections of transmission do not greatly lessen the problem of accounting for the impressive degree of transmission that does occur in the less persistent types.

How then can the hereditary maintenance of cortical variations in the absence of relevant nuclear or endoplasmic differences be understood? Would the assumption that the cortical structures themselves, or any components of them, replicate resolve the problem? The question is most pertinent in regard to the kinetosomes which have been thought to be both self-reproducing and directive or instrumental in morphogenesis. Recent evidences, at the electron microscope level, still leave the matter unsolved. However, even if they reproduced themselves or even if they contained DNA, this would not explain the hereditary maintenance of our cortical variations. These variations are not changes in the kinetosomes or associated structures per se. They are variations in spatial relationships among elementary structures, which themselves appear to be structurally unaltered.

The maintenance of normal or variant patterns of organization can be understood simply in terms of existing structures and the restraints they impose on the development and positioning of new structures. For example, grafted segments of rows of cortical units can be integrated only by being sandwiched between the existing rows. They are forced to grow parallel to them and their only degree of freedom is to have normal or reversed polarities. Once a row is integrated, no degree of freedom is left: as Dippell has shown, new parts, as they arise, are positioned within, and in definite relations to, those of the existing unit.

We have no evidence concerning the molecular nature of such structural constraints or interactions between structures or groups of structures, but it may be worth calling attention to the absence of any necessary theoretical conflict between our results and the implications of the "self-assembly" hypothesis which ascribes the formation of organelles and subcellular structures solely to the properties of their constituent molecules. Our results merely underline a missing and probably decisive component in the system of molecular interactions which should have been recognized a priori, namely, the existing organization that constitutes the milieu where other subcellular organelles and structures are assembled. Newly formed molecules do not enter a vacuum, but a structured cell, the molecules of which are an essential part of the determinism for locating, orienting, and patterning new molecular formations. In this perspective it is conceivable that any subcellular structure might be affected, in the course of its formation, by changes in physical, chemical, or organizational properties of the surrounding intracellular milieu. Some changes may not allow the new structure to be formed at all; but with some probability they may induce a structural alteration in it. A modified structure might in turn, by itself constituting a change in the milieu, no longer permit the formation of the normally patterned structure, but permit reproduction of only the modified form. Such interactions at the molecular level might then result in hereditary extragenic variations.
Whatever the case may be concerning the possible mechanisms of cytoplasmic mutations, our observations on the role of existing structural patterns in the determination of new ones in the cortex of *P. aurelia* should at least focus attention on the informational potential of existing structures and stimulate explorations, at every level, of the developmental and genetic roles of cytoplasmic organization.

**Summary.**—Pieces of cortex of Paramecium can be grafted onto a whole cell and become integrated, yielding a modified cortical pattern which is maintained through both sexual and asexual reproduction. Two types of modified cortical patterns were studied: presence of some rows of cortical units with reversed polarities and presence or absence of whole fields of rows of cortical units. An analysis of the level of fields of rows and at the level of the cortical unit, the organization (presence, location, orientation, and shape) of newly formed structures is determined by the cortical environment existing at the time of their development.

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3 Sonneborn, T. M., these *Proceedings*, 51, 915 (1964).


8 The possession of rows of RP cortical units is doubtless the basis of the twisty swimming behavior, but the relation of the orientation of cortical units to the direction of the ciliary beat is still obscure.


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**GENETIC DAMAGE INDUCED BY ETHYLENIMINE***

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The induction of genetic changes by chemical agents was established by Auerbach and Robson¹ using mustard gas treatment of Drosophila. Before that time, indications of the mutagenic potentialities of chemicals had been obtained in other biological forms, especially plant material.² Further testing with mustard gas re-