including the close proximity of the two chains. The anionic phosphate groups in the adjacent chains are positioned favorably for cations to link the chains together through ionic bonds, in a way similar to the original suggestion by Franklin and Gosling (*6*). Anionic repulsion would surely cause the two chains to be further apart than shown, however.

Several interesting things occur when one physically twists an upper section of the B′ model in a left-handed or unwinding manner. The effect of a small twist is to readily open up a gap between the base pairs adjacent to the twisted and stationary sections of the model. This gap could easily accommodate intercalator molecules such as ethidium bromide or the phenoxazone chromophore of actinomycin D. Larger twists begin to result in the formation of a section of left-handed helix which is quite compatible with the adjacent section of right-handed helix. The complete transition from right- to left-handed helix or vice versa can be accomplished with only 2 bp in the transition region and in a rather confined space, such as in a hydrated crystalline structure.

The regular left-handed helix (Fig. 2c) which forms by twisting the B′ model was adjusted to have axial base pair intervals of 3.32 ± 0.05 Å, the value determined (*8*) for C-DNA. The planes of the base pairs are approximately perpendicular to the helical axis. The helix makes one complete turn for each $9^1/_3$ bp, resulting in a pitch of 31.4 ± 0.2 Å. The radial distance to each phosphorus atom from the helical axis is 9.4 ± 0.3 Å, a value not wholly inconsistent with 9.05 Å proposed from x-ray-diffraction studies (*8*). The sugar residues attached to pyrimidine bases have a C1′ *endo* pucker while sugar residues attached to purine bases have a C3′ *endo* pucker, and both have a nearly *trans* conformation about the C4′–C5′ bond. This model (C′ model) is suggested as an alternative structure for C-DNA. Nonetheless, a right-handed model with $9^1/_3$ bp per turn and acceptable dimensions for C-DNA can also be built. Like the B′ model, the C′ model is relatively smooth and compact, but has an even less distinct minor groove.

Significantly, the right- to left-handed B′ → C′ (also C′ → B′) transition is hindered in those chain segments attached to pyrimidine bases. The O2 atom on a pyrimidine base restricts the sugar rotation during the transition by interfering with hydrogens on C5′ and C3′ (or on C3′ and C5′ for C′ → B′) in the absence of "thermal" chain motions. Thus, each pyrimidine sugar is initially rotated counterclockwise about its glycosidic bond by about 70° less than a purine sugar during the transition. The resulting irregular *syn* chain pattern might lead directly to a form like alternating B- (*16*) or Z-DNA (*13*) in alternating copolymers, or perhaps to an irregular form associated with A-DNA. No regular configuration II models were found which have 11 or more base pairs per turn as in A-DNA (*6*, *7*); however, the study was in no way exhaustive nor were irregular chain models considered.

The configuration II family of models provides a topologically direct path for transitions between right- and left-handed DNA forms. This scheme is consistent with model 2 of Pohl and Jovin (*17*) on the cooperative, reversible, salt-induced transition in the synthetic DNA copolymer having alternating G · C and C · G base pairs. This transition is suggestively referred to as being between R and L forms, since the circular dichroism spectrum inverts in going from one form to the other. In the transition from right-handed B′-DNA to a left-handed form, the activation energy would be associated primarily with the disruption of the stacking interaction between one or more adjacent, intact base pairs.

One final promising result shows that the B′ and C′ models are consistent with the supercoiling of DNA which occurs in chromatin. An outward bend of about 60° toward the almost nonexistent minor groove is readily allowed in the chains lying between each adjacent base pair. The hinge axis is approximately horizontal and passes across the minor groove. As a result, a 180° supercoil bend of these double-helical models can be accommodated over the distance of a small number of nucleotides.

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Hydra Mesoglea: A Model for Investigating Epithelial Cell–Basement Membrane Interactions

Abstract. *Isolated hydra mesoglea served as a suitable substrate for the attachment and spreading of hydra cells in vitro, irrespective of the species tested. Hydra cells did not attach and spread on substrates typically used for culturing mammalian cells. Mammalian and* Drosophila *cells attached and spread on plastic culture dishes but not on isolated mesoglea.* Xenopus *epithelial cells spread on both plastic and mesoglea. Because of the similarities of hydra mesoglea to vertebrate basement membranes, suggestions are offered for using mesoglea to study the interactions of epithelial cells with their basement membranes.*

The extracellular matrix influences a number of normal cellular and developmental activities, such as cell migration, differentiation, and proliferation (*1*, *2*). It has also been implicated in abnormal cellular behaviors, including neoplasia (*2*). The matrix exists as a primitive basement membrane in the freshwater hydra, where it is sandwiched between the two epithelial layers that make up the animal. Known as the mesoglea, it has physical and biochemical similarities to the basement membrane of vertebrates (*3*). We used isolated mesoglea to study the interactions of epithelial cells with an extracellular matrix in vitro.

Intact mesogleas totally free of adhering cells were isolated from cultures of *Hydra attenuata* (*4*) by a modification of the technique of Barzansky *et al.* (*5*): a 0.1 percent solution of the detergent Nonidet P-40 was substituted for Sarkosyl NL-97. The isolated mesoglea retained the hydroid shape of the polyp (Fig. 1a) and often exhibited a rectilinear fiber system (*6*) under phase-contrast mi-

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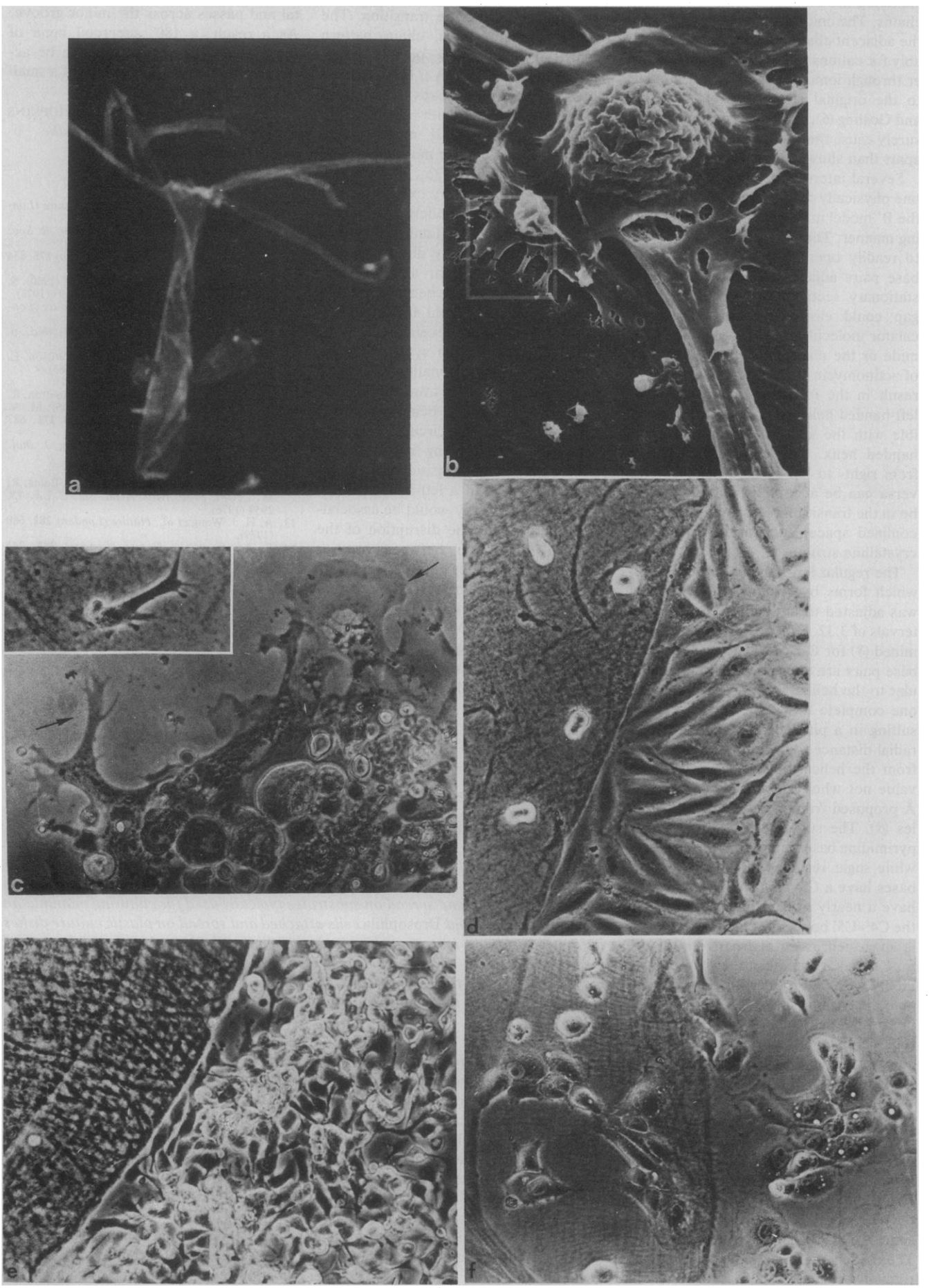
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Table 1. Attachment and spreading of hydra cells on various substrates. Symbols: +, consistently positive, ±, inconsistent or marginal; 0, consistently negative.

Substrate	Attachment	Spreading
Mesoglea	+	+
Plastic (culture dish)	0	0
Glass	0	0
Gelatin	0	0
Fetal calf serum	0	0
Collagen	0	0
Fibronectin (bovine)	±	0
Concanavalin A	+	0
Wheat germ agglutinin	+	±
Poly-L-lysine	+	±

Table 2. Attachment and spreading of hydra cells on mesoglea from different hydra species. Two plus signs denote a larger number of cells attaching than one plus sign.

Cell	Mesoglea	Attachment	Spreading
H. attenuata	*H. viridis** (English aposymbiont)	+	+
H. attenuata	*H. viridis* (English green)	+	+
H. attenuata	*H. viridis* (Florida aposymbiont)	++	+
H. viridis (Florida aposymbiont)	*H. attenuata*	+	+
H. attenuata	*Hydra* sp. (Carolina)	++	+
Hydra sp. (Carolina)	*H. attenuata*	++	+

**Hydra viridis* normally appears green due to the presence of symbiotic algae within the endodermal cells. Aposymbionts are clones derived from specimens of *H. viridis* that are free of algae. Results of experiments in which cells from *H. viridis* (English green and aposymbiont) were used were difficult to interpret. These animals were raised at 10°C and did not adjust well to room temperature (21° to 23°C), the culture and experimental temperature for the other animals.

croscopy (Fig. 1e). Mesogleas stored at −65°C in distilled water were thawed and transferred to 35-mm-diameter Falcon plastic culture dishes. The mesogleas settled to the bottom in the dishes and adhered there after excess water was removed with a finely tapered Pasteur pipette.

Suspensions of live hydra cells, both single and in small clumps, were prepared in a 95-milliosmolar disaggregation solution (*7*). Hydra cells placed in this solution, which has approximately 15 times the solute concentration found in hydra culture medium, are still alive after 24 hours, as determined by trypan blue exclusion. The suspensions, prepared from 300 to 500 hydra, were placed in the culture dishes, whose bottoms had been coated with one of the substances listed in Table 1. In addition to mesoglea, we used a number of substrates known to promote the adhesion and spreading of mammalian cells (*8*, *9*). The cells were usually allowed to settle for 30 to 45 minutes, after which the suspensions were gently swirled to dislodge loose cells. These cells were removed with a pipette, and the dishes were rinsed and replenished with fresh disaggregation solution. The dishes were then placed on an inverted phase-contrast microscope (Leitz Diavert) and examined. We found that the hydra cells consistently attached and spread only on hydra mesoglea (Table 1).

Cells contacting mesoglea began to adhere within minutes, whereas cells settling on plastic did not adhere at all (*10*). Active attachment to the mesoglea was usually observed at the peripheries of clumps of 10 to 25 cells. Although it was difficult to identify the types of cells attaching, most appeared to be epithelial. In 30 to 60 minutes, many of the attached cells on the peripheries of the clumps began to spread (Fig. 1c). Filopodia as well as broader lamellipodia protruded from the cells onto the mesoglea (Fig. 1b) and sometimes onto the surrounding plastic (Fig. 1c). Eventually the remaining cells of an attached clump also flattened out.

To determine whether the observed attachment and spreading of hydra cells on the mesoglea was species-specific, we mixed hydra cells and mesoglea from different species and strains. Cells from *H. attenuata* attached and spread on the mesoglea of each hydra strain tested. More cells stuck to the mesoglea of some hydras than others (Table 2). Cells from *H. viridis* and *Hydra* sp. (Carolina) attached to and spread on *H. attenuata* mesoglea. Thus, it appears that the cells of the hydras tested can attach to mesoglea from other hydras irrespective of their origin.

Because mesoglea served as an excellent substrate for hydra cells to attach to and spread on, cell suspensions from a variety of other sources were tested for their ability to use mesoglea as a substrate. We tested three mammalian cell types (marsupial kidney Pt K2, murine B16-F1 melanoma, and nonmuscle cells from rat heart), one amphibian cell line (*Xenopus* A6), and one insect cell line (*Drosophila* KspA 1-1). All were tested in their standard culture media.

Although all the mammalian cells spread readily on the plastic dishes containing mesoglea, only a few attached to the mesoglea. Of these cells, fewer still showed limited spreading. The Pt K2 and rat heart cells spread as a monolayer on the plastic adjacent to the flattened mesoglea; it was not clear whether the cells contacted the mesoglea itself (Fig. 1d). The B16-F1 cells, which characteristically form piles of cells in culture instead of a monolayer (*11*), also spread up to the mesoglea without clearly contacting it (Fig. 1e). Individual mammalian cells that were attached to the plastic did not send out processes onto the mesoglea.

The insect cells behaved similarly, attaching in large numbers to the plastic but not the mesoglea (*12*). The amphibian A6 cells appeared to attach and spread on the mesoglea, although not as extensively as on the plastic (Fig. 1f). In addition, individual A6 cells extended processes across the mesogleal border, spreading from one substrate to the other.

These results may provide insight into how epithelial cells in general recognize and interact with their natural substrate, the basement membrane. By treating the mesoglea in various ways it may be possible to determine the nature and orientation of the components that are recognized by the cells and cause them to attach and spread. By manipulating the mesoglea we may learn how the extracellular matrix affects cell shape, motility, and behavior. In hydra it may be possible to test whether the mesoglea orients the muscle processes of the epithelial cells, thereby affecting the shape and form of the animal.

Our findings have particular importance to researchers using hydra as a model system for studying developmental biology. For example, it would be desirable to culture individually some of the seven main hydra cell types. Past attempts at culturing hydra cells were unsuccessful (*13*, *14*). A major difficulty has been in getting disaggregated hydra cells

Fig. 1. (a) Intact mesoglea of *H. attenuata* retaining its hydroid shape, including tentacles and buds (×10). (b) Scanning electron micrograph of a hydra cell spread out on an isolated mesoglea (×4000). The cell processes seem to be merging with the mesoglea (box). (c) A clump of hydra cells showing peripheral cells attaching and spreading onto the plastic culture dish (×1600). Arrows point to filopodia and lamellipodia. Inset: a single cell spreading on mesoglea (×800). (d) Monolayer of Pt K2 cells (right) spreading on plastic next to mesoglea (×800). The few cells on the mesoglea are rounded and have neither attached nor spread. (e) Mouse B16-F1 melanoma cells (right) piled on one another next to the mesoglea (×850). Note the rectilinear arrangement of the mesogleal fibers. (f) *Xenopus* A6 cells spreading on both plastic (right) and mesoglea. Note the fibers extending past the mesogleal border (×800).

to attach and spread on a substrate. Because most nontransformed animal cells must attach to a substrate before they can grow (*9*, *15*), the failure to culture hydra cells may have resulted in part from the use of unsuitable substrates. The use of mesoglea as a substrate for isolated hydra cells might represent, therefore, a major step toward their successful culture.

Mesoglea is probably not a prime factor in causing graft rejection (Table 2). For example, cells from *H. attenuata* stick and spread on mesoglea produced by *H. viridis* epithelial cells but will not form cell junctions with the cells themselves; grafts between portions of individuals of the two species separate in situ (*16*). Hence the cause of graft rejection between different hydra species may lie not with cell-mesogleal interactions but rather with cell-cell interactions.

In contrast to hydra cells, mammalian and *Drosophila* cells did not find hydra mesoglea suitable for attachment and instead tended to stay rounded when contacting the mesoglea. When the dish was agitated, the cells rolled onto the surrounding plastic and spread there (Fig. 1, d and e), as though avoiding the mesoglea. Just as the mammalian cells did not attach well to the mesoglea, hydra cells did not attach and spread on substrates suitable for most mammalian cells (Table 1). The amphibian cells, on the other hand, stuck and spread well on both mesoglea and plastic.

Should we expect mammalian cells to attach to an extracellular matrix produced by an animal as primitive as the hydra? Animals from the phylum Cnidaria were the first to evolve epithelia, and the mesoglea may represent an early progenitor of vertebrate basement membranes. Hydra mesoglea, for example, is homologous to vertebrate basement membrane in a number of ways (*3*, *5*): it contains a collagen-like component, as evidenced by its amino acid composition and the presence of a glucosylgalactose disaccharide unit; there is an increase in the amounts of hydroxylysine and neutral sugars relative to vertebrate collagen; and it is continually secreted by the overlying epithelial layers. If the vertebrate basement membrane did evolve from the mesoglea, we may be able to use the latter as a model to determine which components or arrangement of components are recognized by epithelial cells.

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Giant Synaptic Potential Hypothesis for Epileptiform Activity

Abstract. *According to one hypothesis, the paroxysmal depolarizing shift observed in the penicillin model of epilepsy results from a giant excitatory postsynaptic potential. This hypothesis has recently been questioned, primarily because it has never been subjected to rigorous experimental examination. Four quantitative predictions were derived from this hypothesis and tested in CA3 pyramidal neurons of the hippocampus. The four critical predictions concern the behavior of the paroxysmal depolarizing shift under current- and voltage-clamp conditions as a function of membrane potential. The experiments confirmed all four predictions.*

One of the most extensively studied animal models of epilepsy is provided by the topical application of penicillin to the mammalian cortex (*1*–*4*). Periodic interictal epileptiform discharges are recorded in the electrocorticogram after this treatment. The intracellular correlate of these interictal events consists of a sudden 20- to 50-mV depolarization, which lasts for 50 to 100 msec. This sudden depolarization was originally termed a paroxysmal depolarization shift (PDS) by Matsumoto and Ajmone-Marsan (*1*). The mechanism underlying the PDS has been extensively studied because it is believed to bear directly on our understanding of the cellular basis of epilepsy. The most influential hypothesis regarding the mechanism of PDS generation is that the PDS is a giant compound excitatory postsynaptic potential (EPSP) (*2*). According to this hypothesis, penicillin increases the efficacy or recruitment of recurrent excitatory circuits, which in turn are responsible for the giant EPSP. The giant EPSP hypothesis does not specify the mechanism whereby penicillin has this effect on recurrent excitation, but it might be imagined to occur by a reduction in feed-forward or recurrent inhibition, since we have recently demonstrated that penicillin blocks all detectable inhibitory miniature synaptic potentials in hippocampal neurons (*5*).

The usual evidence for and against the giant EPSP hypothesis is indirect and unconvincing (*2*–*4*). We therefore derived and tested certain quantitative predictions of the central tenet of this hypothesis; namely, that the PDS is a very large EPSP. The first prediction is that the frequency or probability of occurrence of PDS's should be unaltered by changes in the membrane potential. By contrast, if the PDS were purely an intrinsic regenerative membrane event, its frequency or probability of occurrence should be affected by changing the membrane potential. Second, if the PDS is a large EPSP, its amplitude should be a monotonically decreasing function of the membrane potential in accordance with the decrease in synaptic driving force. Third, if the PDS is synaptic in origin, it should be possible to reverse its polarity by depolarizing the cell beyond the synaptic equilibrium potential. Fourth, if the PDS is a *giant* EPSP, then the synaptic currents underlying the PDS should be large relative to those accompanying normal spontaneously occurring EPSP's. A set of similar quantitative predictions can be derived for the assumption that the PDS is a large EPSP caused by a decrease in conductance (*6*).

Until recently, it was not possible to evaluate these predictions experimentally in mammalian cortical neurons, since an adequate test requires changing the membrane potential to extreme voltages under both current- and voltage-clamp conditions. We have developed methods

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