of 10 to 50 μm. Sixteen to 22 percent of these cells formed the presumed gamma group, with mean diameter 19 μm. Postoperatively, however, only 2 to 3 percent of labeled cells were this small. This discrepancy is unlikely to result from posttraumatic swelling of the anterior horn cell, since the disappearance of small cells was not accompanied by increase in maximum cell size (*14*). Brown and Butler (*15*) and Thulin (*16*) have provided electrophysiologic evidence for the return of gamma function in the cat after the nerve to the tenissimus muscle was crushed or a 1-cm segment of posterior tibial nerve was resected. However, Takano was unable to demonstrate gamma reinnervation of muscle spindles 6 months after local freezing of the cat sciatic nerve (*17*). In Brown and Butler's and in Thulin's experiments, nerve was disrupted near its termination; in Takano's and ours, however, the injury was midway between spinal cord and muscle. The failure of gamma reinnervation after sciatic injury may thus be attributed to either (i) the inability of gamma motoneurons to regenerate over relatively long distances or (ii) the allowance of insufficient time for regeneration to occur.

The importance of gamma regeneration has not been established. Takano demonstrated a return of normal gait in cats after freezing the sciatic nerve, even though gamma reinnervation could not be demonstrated (*17*). Conversely, Thulin showed that, after posterior tibial nerve injury, push-off in gait did not occur with adequate gastroc-soleus strength until gamma reinnervation was reestablished (*16*). More research is needed to define the patterns and functional consequences of gamma reinnervation after various types of injury.

We have demonstrated alterations in the quantity, position, and size distribution of the anterior horn cells serving a muscle group after repair of the innervating multifascicular nerve. Mark has summarized the deficits following nerve severance and repair in higher vertebrates as loss of coordinated movement with capacity for only graded mass contraction (*1*). He suggests that undirected growth of axon sprouts has led many axons to the wrong muscle, so that a pool of motoneurons that previously served one muscle controls motor units scattered among several. Demands for contraction of the original muscle result in weak contraction of the whole group. We have demonstrated the anatomical changes Mark hypothesized and provided evidence for alterations in the gamma control mechanism. However, the functional consequences of these changes will depend on the quality of sensory regeneration and the degree of central adaptation to anterior horn disorganization. Combined anatomic and physiologic investigation will be necessary to assess the contribution of each to the end results of nerve repair.

THOMAS M. BRUSHART
M.-MARSEL MESULAM*
Neurology Unit, Beth Israel Hospital, Boston, Massachusetts 02215

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* Reprint requests should be addressed to M.-M.M.

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Bladder-Surface Glycosaminoglycans: An Efficient Mechanism of Environmental Adaptation

Abstract. *The transitional epithelium of the urinary bladder secretes and binds to its surface a glycosaminoglycan that inhibits the adherence of bacteria. Synthetic sulfonated glycosaminoglycans instilled intraluminally into bladders whose natural mucin layer has been removed are as effective as the natural mucin in preventing bacterial adherence. It also appears that adherence of calcium and protein is reduced in the presence of both the natural mucin layer and the synthetic sulfonated glycosaminoglycan sodium pentosanpolysulfate, suggesting that the antiadherence activity of both natural and synthetic surface glycosaminoglycans in the bladder extends to the molecular and ionic levels.*

The urinary bladder endosurface faces a relatively hostile environment, one that contains high levels of calcium, potential carcinogens, and at times pathogenic microorganisms. Our previous studies showed that the transitional cell epithelium produces and maintains at its surface a glycosaminoglycan whose presence may explain why the bladder surface is so resistant to these insults (*1-9*). The presence of glycosaminoglycan is associated with a marked impairment of the ability of bacteria to adhere to the surface. Furthermore, it is possible to remove the natural glycosaminoglycan and reproduce its antiadherence effect in vivo with several synthetic glycosaminoglycans (*3, 4, 9*). These findings suggested a new mode of antibacterial therapy in which a natural immune mechanism is augmented. We conducted the experiments reported here in order to determine whether the antiadherence effect of natural and synthetic glycosaminoglycans extends to the molecular and ionic levels, a phenomenon that would open the possibility of many new therapeutic uses for the synthetic compounds.

The assay for measuring bacterial adherence to the bladder mucosa of the rabbit in vivo was described in detail by Parsons and Mulholland (*5*). We used the same assay in the present studies, except that ^{45}Ca or ^{14}C-labeled protein was substituted for bacteria.

Male New Zealand White rabbits (2 to 3 kg) were given, via penile catheter, ^{45}Ca (0.1 μCi; New England Nuclear) or ^{14}C-labeled protein (0.1 μCi; Amersham), each suspended in 0.5 ml of physiological saline solution (PSS). Group 1 (control) rabbits, whose bladder mucin

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layer was left intact, received 0.5 ml of PSS followed immediately by the ^{45}Ca or ^{14}C-labeled protein. In group 2 rabbits, the bladder mucin layer was removed with acid (*5*) and 0.5 ml of PSS was injected into the bladders immediately before the addition of ^{45}Ca or ^{14}C-labeled protein. In group 3 rabbits, the bladder surface mucin was removed with acid, and 0.5 ml of sodium pentosanpolysulfate (10 mg/ml; Benechemie) was added, followed immediately by the ^{45}Ca or ^{14}C-labeled protein. The bladders of group 4 rabbits were also acid-treated to remove surface mucin and then were injected with 10 mg of sodium pentosanpolysulfate in 1 ml of PSS. After 30 minutes, this solution was removed, the bladders were rinsed with two 15-ml portions of PSS, and 0.5 ml of PSS and the ^{45}Ca were added to the empty bladders.

In all groups, after the ^{45}Ca or ^{14}C-labeled protein had been allowed to interact with the bladder epithelium for 15 minutes, 10 ml of PSS was injected into the bladders to dilute the calcium or protein and terminate the reaction. The penile catheters were then opened to provide straight drainage. After the rabbits had each produced 50 to 70 ml of urine, they were killed and their bladder mucosas were removed and prepared for radioactivity assay (*5*).

Two types of experiments were conducted; in both six rabbits were used (either two controls, two acid controls, and two treated simultaneously with acid and sodium pentosanpolysulfate; or two controls, two acid controls, and two treated with sodium pentosanpolysulfate before being exposed to acid). Using Student's *t*-test, we subjected all data obtained to an analysis of variance and the rank-sum test. Differences in mean radioactive protein or calcium adherence values were considered significant when $P < .05$ by both tests.

As shown in Table 1, adherence of ^{45}Ca to the mucosal surface was 3.5 times greater in acid-treated bladders than in untreated control bladders ($P < .001$). When mucin-deficient bladders received sodium pentosanpolysulfate simultaneously with the calcium, such a rise in adherence was not seen. Bladders were also protected from an increase in calcium adherence when they were first treated with sodium pentosanpolysulfate and rinsed free of the synthetic glycosaminoglycan before being exposed to the calcium solution (Table 2).

As shown in Table 3, the adherence of ^{14}C-labeled protein to mucin-deficient bladders was 20 times higher than the adherence to control bladders ($P < .001$). When sodium pentosanpolysulfate was added to mucin-deficient bladders immediately before the ^{14}C-labeled protein was added, protein adherence dropped nearly to control levels.

The surfaces of the transitional cells in the urinary bladder must interact with high, often supersaturated levels of calcium, compounds potentially capable of providing a matrix for calcium deposition, possible carcinogens, and pathogenic microorganisms. We believe that a relatively simple but effective mechanism enables the bladder surface to maintain its equilibrium in the face of these challenges.

Table 1. Effects of natural bladder mucin and sodium pentosanpolysulfate on adherence of ^{45}Ca to bladder epithelium. The data are expressed as means ± standard deviations.

Experimental group	^{45}Ca (count/min per milligram of mucosa × 10)
Control (N = 21)	3.6 ± 2.4
Acid control (N = 24)	12.4 ± 8.7*
Acid plus sodium pentosanpolysulfate	
20 mg/ml (N = 10)	3.5 ± 2.9
10 mg/ml (N = 11)	4.0 ± 3.2
5 mg/ml (N = 15)	3.7 ± 2.9

*$P < .001$.

Table 2. Adherence of ^{45}Ca after first treating bladder with sodium pentosanpolysulfate (10 mg/ml). The data are expressed as means ± standard deviations.

Experimental group	^{45}Ca (count/min per milligram of mucosa × 10)
Control (N = 25)	5.4 ± 5.8
Acid control (N = 20)	17.3 ± 12.1*
Sodium pentosanpolysulfate (N = 27)	8.8 ± 5.5

*$P < .001$.

Table 3. Effects of natural bladder mucin and sodium pentosanpolysulfate (5 mg/ml) on adherence of protein to the bladder epithelium. The data are expressed as means ± standard deviations.

Experimental group	^{14}C-labeled protein [milligrams per milligram of mucosa (dry weight)]
Control (N = 16)	3.3 ± 3.5
Acid control (N = 20)	28.7 ± 31.1*
Sodium pentosanpolysulfate plus protein† (N = 15)	5.5 ± 6.8

*$P < .001$. †Bladder mucin layer first removed with acid.

When we began our studies, the resistance of the bladder to infection was well known but had not been explained. We previously discussed the work of investigators whose studies of the gastrointestinal tract, oral cavity, and genitourinary tract have centered on the concept that bacterial adherence is a prerequisite to infection (*3, 4*). If bacterial adherence is a prerequisite to infection, the host must have defense mechanisms capable of interfering with it. It has been suggested that immunoglobulin A and glycoproteins inactivate bacterial adherence factors such as pili or glycocalyx in the manner of an antigen-antibody reaction; however, we feel that because of its extreme specificity such a mechanism would not explain the bladder's resistance to the myriad of microorganisms against which there would be no preformed antibodies.

By measuring adherence in the rabbit bladder (*1*) and employing histochemical techniques, we found that the transitional epithelium secretes a glycosaminoglycan whose presence on the cell surface reduces the ability of bacteria to adhere to the mucosal cells by 50 to 60 times (*1, 2, 5, 7, 8*). Our data showed that bladders whose natural mucin had been removed with acid are totally protected from an increase in bacterial adherence if synthetic glycosaminoglycan is placed intraluminally immediately before bacteria are added. The synthetics appear to act as a barrier to adherence by coating the bladder wall (*3, 4*).

Beginning with this concept, we formulated a detailed hypothesis for the action of glycosaminoglycans in preventing adherence (*3, 4*). Researchers in the field of synthetic membrane technology have defined important qualities of sulfonated glycosaminoglycans, one of which is their extremely hydrophilic nature. Each sulfonated moiety of the compound will bind several water molecules very tightly, in effect "wetting" the surface. In the urinary bladder, glycosaminoglycan could place a water barrier between the transitional cells and the environment and thus mask highly charged moieties on the cell surfaces. This barrier would serve to interfere with electrochemical reactions, thereby impairing bacterial binding.

Such a mechanism should also block the electrochemical adherence of many other substances. We tested this by substituting radioactively labeled calcium or protein for bacteria in our adherence assay in vivo. We found that the adherence of both calcium and protein was significantly reduced in the presence of the sur-

face glycosaminoglycan. We also found that sodium pentosanpolysulfate prevented the adherence of calcium and protein to bladders whose natural mucin had been removed. To determine whether sodium pentosanpolysulfate was preventing adherence to the transitional cells by binding the calcium, we treated bladders with sodium pentosanpolysulfate for 30 minutes, rinsed it free, and then instilled the calcium. Adherence of calcium to the bladder epithelium was prevented under these circumstances as well (Table 2).

The concept of bladder-surface glycosaminoglycan as an antiadherence factor active at the cellular, molecular, and ionic levels offers an appealing model of a simple and efficient mechanism by which the bladder might deal with its environment. This mechanism, not limited by the specificity of an antigen-antibody reaction, would enable the bladder to dispose easily of a variety of microorganisms. At the same time, it would prevent calcium, which is often supersaturated in urine, from adhering to the surface and acting as a nidus for stone formation. Furthermore, this mechanism would prevent the adherence of other molecules that could act as a matrix for calcium deposition. It is possible that bladder-surface glycosaminoglycan reduces the interaction of potential carcinogens with the transitional cells.

The urinary tract may not be the only mucous surface in the body to rely on sulfonated glycosaminoglycans as antiadherence factors. The surface of the eye is lined with a heparinlike substance (*10*), and commercial heparin is purified from the gastrointestinal tract of the pig. Endothelial cells of blood vessels also synthesize heparin, which lines the blood vessel walls (*11*, *12*); the advantages of the presence of an antiadherence factor in the vascular system are obvious.

C. LOWELL PARSONS
CHARLES STAUFFER
JOSEPH D. SCHMIDT
Urology Section, San Diego Veterans Administration Hospital, La Jolla, California 92161, and *Division of Urology, Department of Surgery, University of California Medical Center, San Diego 92103*

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Olfactory Sensitivity in Humans: Genetic Versus Environmental Control

Abstract. *Olfactory sensitivity to acetic acid, isobutyric acid, and 2-sec-butyl-cyclohexanone was tested in 97 adult male twin pairs to determine the extent to which variation in odor perception was genetically determined. Analysis of the data revealed no evidence for heritability of olfactory sensitivity. However, factors significantly associated with odor perception included cigar, pipe, and cigarette smoking; body fatness; alcohol consumption; and diabetes mellitus.*

Current theories of sensory perception in humans suggest that variation in the ability to detect odors may have a significant genetic component. General sensitivity to odors is thought to be influenced by congenital conditions and by the endocrine status of the body (*1*). Specific sensitivity to an odor may be determined by the functional group, molecular size, or molecular shape of the odorant (*2*, *3*). It has been proposed that perception results from the "fit" of an odorant to receptor sites in the olfactory organ (*2*, *3*). A logical assumption is that variation in the structure of the olfactory receptors may be genetically controlled (*4*). Although observed threshold distributions (*4*, *5*) are compatible with genetic mechanisms, and insensitivity to particular odors (that is, specific anosmias) clusters in families (*6*), little is actually known about heritability over the range of odor sensitivity.

A unique opportunity to investigate genetic variability of olfactory sensitivity arose at the inception of the National Heart, Lung, and Blood Institute (NHLBI) twin study. Participants in this multicenter study were male veteran twins, aged 42 to 56, who were ascertained from the National Academy of Sciences–National Research Council Twin Registry (*7*) and who volunteered to undergo a physical examination at the invitation of the NHLBI (*8*). Tests of olfactory sensitivity were successfully administered to 97 twin pairs, 51 monozygotic (MZ) and 46 dizygotic (DZ), examined at the Framingham, Massachusetts, study center.

Twin populations are ideal for studying the extent to which variation in a characteristic may be genetically determined. Classical twin analysis (*9*, *10*) attempts to relate the similarity between MZ twins, who share all of their genes, to the similarity between DZ twins, who share, on the average, 50 percent of their genes. In this study, evidence for genetic control of odor perception existed if, under certain basic assumptions, olfactory sensitivity was more similar (more highly correlated) for MZ than DZ twin pairs.

An individual's olfactory sensitivity was determined by exposing him to serial dilutions of three chemical compounds thought to represent different "primary" odors as described in Amoore's early stereochemical theories (*3*, pp. 96–125; *5*). The compounds used were acetic acid, isobutyric acid, and 2-*sec*-butyl-cyclohexanone (cyclohexanone), belonging

Table 1. Summary of the twin analysis for olfactory sensitivity. Results are adjusted for examination time. The number of twin pairs is shown in parentheses. Estimates of genetic variance using the method of Christian *et al.* (*10*) were not significant and therefore are not shown.

Chemical compound	Intraclass correlations (r) of sensitivity thresholds*	
	r_{MZ}	r_{DZ}
Acetic acid	0.38 (48)	0.11 (43)
Isobutyric acid	0.23 (47)	0.41 (43)
Cyclohexanone	0.10 (50)	0.04 (44)

*To test the hypothesis that $r_{MZ} = r_{DZ}$, the following normal statistic was used:

$$z = \frac{z_{MZ} - z_{DZ}}{\sqrt{\sigma^2_{MZ} + \sigma^2_{DZ}}}$$

where

$$z_{MZ} = .5\left(\log_e \frac{1 + r_{MZ}}{1 - r_{MZ}}\right)$$

$$z_{DZ} = .5\left(\log_e \frac{1 + r_{DZ}}{1 - r_{DZ}}\right)$$

$$\sigma^2_{MZ} = \frac{1}{N_{MZ} - 3} \quad \sigma^2_{DZ} = \frac{1}{N_{DZ} - 3}$$

and N = number of twin pairs.

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