the establishment or the maintenance of the amoebal state, or both. Since the ALC plasmodia were obtained by screening plasmodia formed from heterothallic amoebae, it seems likely that the mutation causes at least a defect in the maintenance of the amoebal state. The results of the heterokaryon experiment are also compatible with a maintenance defect. The occasional amoebae that arise in platings of ALC spores may be due to reversion, suppression or leakiness of an ALC mutation. If they are due to suppression or leakiness, it will be possible to use them in further genetic analysis of the ALC defect. Preliminary results indicate that three ALC strains yield only phenotypically true revertants, one ALC strain yields only amoebae that are temperature sensitive for growth and form plasmodia in clones, and one ALC strain yields amoebae of more than one phenotype. Thus, we will be able to analyze further at least two of the ALC mutants.

Henny (*10*), studying progeny of a cross between two natural isolates in *Physarum flavicomum*, found spores that seemed to give rise to plasmodia without any evidence of amoebae. In *P. polycephalum*, spores of sexually produced plasmodia occasionally yield amoebae that are heterozygous for *mt* (*4*), and these amoebae form plasmodia at a very high rate. Since the *P. flavicomum* spore germination was not observed microscopically, the apparent plasmodia-producing spores may actually have been mating type heterozygotes.

The ALC variants may prove useful for obtaining plasmodial mutants. Since we have seen plasmodia arise from normal-sized spores, it is likely that such plasmodia are "cloned" from a single nucleus. Large ALC plasmodia could be treated with a mutagen and made to undergo sporulation as the first step in obtaining mutants. Then the spores could be germinated and selection or screening performed on the resulting small plasmodia. This technique would obviate the current need (*7*) of mutagenizing amoebae and picking plasmodia formed from amoebal clones for later screening.

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Vasopressin: Induced Structural Change in Toad Bladder Luminal Membrane

Abstract. *Freeze-fracture electron microscopy demonstrates that vasopressin stimulation of isolated toad bladder alters the structure of the luminal membrane of granular cells. This alteration consists of an ordered aggression of intramembranous particles, and appears to be of functional significance, since the frequency of aggregation sites per area of membrane is closely correlated with vasopressin-induced osmotic water flow.*

The enhanced water permeability of the isolated toad urinary bladder in response to vasopressin is well established (*1*). The mechanism of this response appears to involve both vasopressin stimulation of cyclic adenosine monophosphate formation within epithelial cells of the toad bladder and subsequent alteration of the luminal membrane of these cells (*2*). While many details of this model have not yet been re-

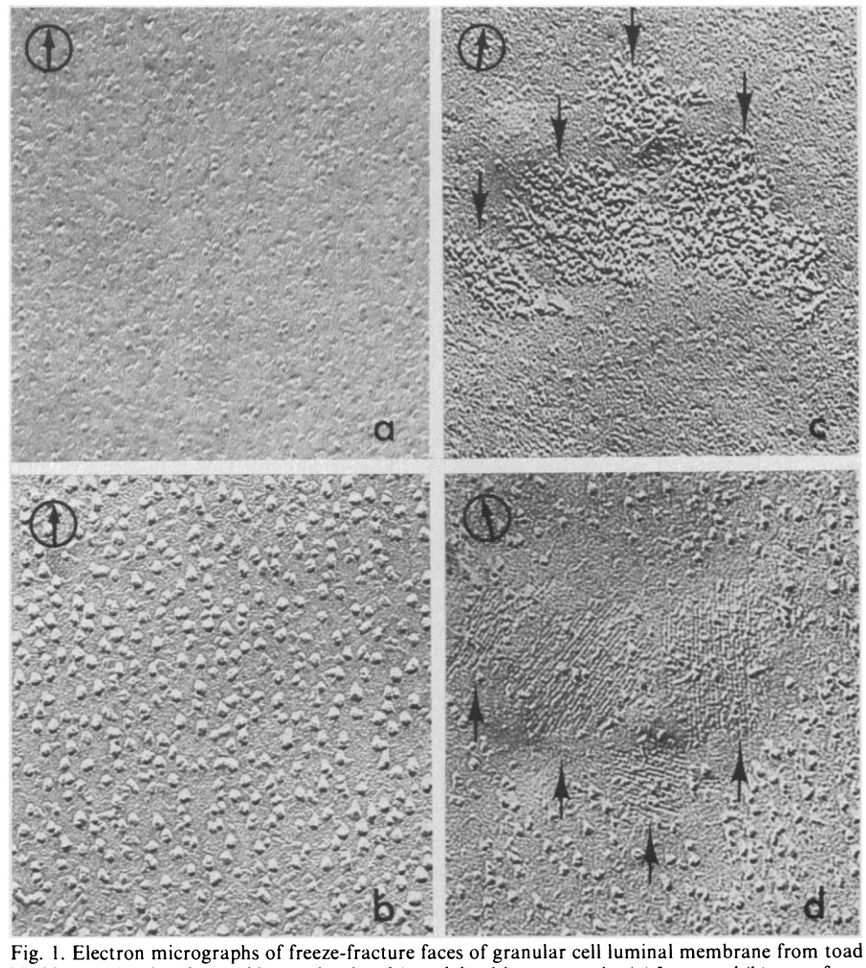

Fig. 1. Electron micrographs of freeze-fracture faces of granular cell luminal membrane from toad bladder unstimulated (a and b) or stimulated (c and d) with vasopressin. (a) Inner and (b) outer fracture faces; without vasopressin stimulation intramembranous particles are not aggregated. (c) Inner fracture face after vasopressin stimulation; separate sites of aggregated intramembranous particles are emphasized (single arrows). (d) Outer fracture face which is complementary to that shown in (c) after vasopressin stimulation; organized linear arrays of depressions corresponding to the aggregated intramembranous particles shown in (c) are emphasized (single arrows). Circled arrows indicate shadowing direction (× 83,000).

solved, it is clear that vasopressin induces an alteration in the water permeability of the luminal membrane of granular cells specifically (*3*). This report presents evidence that in toad bladder vasopressin induces a specific structural alteration of the granular cell luminal membrane demonstrable by freeze-fracture electron microscopy. Furthermore, this alteration appears to be of functional significance, since it is quantitatively associated with vasopressin-induced osmotic water flow. A related structural alteration in frog urinary bladder due to oxytocin exposure has recently been described by Chevalier *et al.* (*4*).

Paired hemibladders from double-pithed female toads (*Bufo marinus*), obtained from the Dominican Republic, were mounted as sacs. One served as an experimental bladder, the other as a reference-control. They were bathed at room temperature (22° to 24°C) on the serosal surface with 200 ml of aerated Ringer solution (111 m*M* NaCl, 3.5 m*M* KCl, 2.5 m*M* $NaHCO_3$, 1.0 m*M* $CaCl_2$; *p*H 7.6 to 8.2; 220 milliosmols per kilogram of H_2O) and on the mucosal surface with Ringer solution diluted 1 : 5 with distilled H_2O. After a 30-minute stabilization period, baseline osmotic water flow was measured gravimetrically in both bladders for 30 minutes (*5*). Immediately thereafter a 30-minute experimental period began, during which osmotic water flow was measured. The reference-control bladders were placed in a 200-ml bath of aerated Ringer solution containing 20 milliunits of vasopressin per milliliter (Pitressin, Parke-Davis) in order to induce a maximal response in osmotic water flow. The experimental bladders were placed in a similar bath containing concentrations of vasopressin selected to give a range of intermediate water flow responses compared to the reference-control bladders. At the end of the experimental period bladders were fixed for 15 minutes in 2.5 percent glutaraldehyde containing 0.1*M* cacodylate buffer (*p*H 7.4) and stored at 4°C in 0.1*M* cacodylate buffer. Vasopressin-stimulated water movement was calculated from weight loss during the experimental period in excess of baseline loss and standardized for surface area which was estimated from mucosal volume.

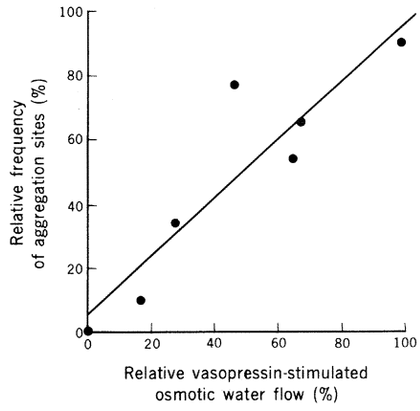

Fig. 2. The relationship between relative vasopressin-induced osmotic water flow and relative frequency of sites of aggregated intramembranous particles. Least-squares regression line: $Y = 5.17 + 0.92X$ ($r = .91$; $P < .005$).

Tissue samples were coded so that freeze fracture, electron microscopy, and evaluation of electron micrographs were done without knowledge of treatment. After soaking in 25 percent glycerol containing 0.1*M* cacodylate buffer to prevent ice crystal formation, tissues were frozen at −150°C in liquid Freon 22 cooled by liquid nitrogen. Freeze fracture was done and replicas of complementary fracture faces were made with a Balzers freeze-etch unit (BAF 301), equipped with an electron beam evaporation device (EVM 052), quartz crystal thin film monitor (QSG 201), and a mirror image replica device. Complementary tissue replicas were examined with an RCA electron microscope (EMU 4B) at 75 kv.

With the freeze-fracture technique membranes are fractured within their hydrophobic interior (*6*), and two fracture faces are produced: a fracture face A, or inner membrane face, and a fracture face B, or outer membrane face. The structure of the granular cell luminal membrane revealed by freeze fracture of a bladder unstimulated by vasopressin is shown in Fig. 1, a and b, and is in accord with the detailed description given by Wade *et al.* (*7*). The same membrane type from a bladder exposed to vasopressin is also shown in Fig. 1, c and d. Unlike the control, it is characterized by the presence of distinctive and organized sites of aggregated intramembranous particles. These particle aggregations are found only in granular cell luminal membranes and not in membranes of adjacent mitochondria-rich or goblet cells. The organization of aggregation sites appears often to consist of parallel rows of tightly packed intramembranous particles on fracture face A (Fig. 1c), and this organization is clearly confirmed by parallel rows of depressions found on the complementary fracture face B (Fig. 1d).

In other tissues aggregation of intramembranous particles has been associated with certain levels of acidity (*8*), cold (*9*), and cryoprotectant treatment without fixation (*10*). Although our findings cannot be attributed to any of these conditions, it is important to point out that the intramembranous particles which are aggregated by these conditions appear to lack consistent organization. Our observations indicate that in response to vasopressin stimulation aggregated intramembranous particles are invariably organized into parallel rows. Accordingly, we interpret these regions to represent a specific vasopressin-induced reorganization of membrane structure.

To determine whether the structures we have described are related to vasopressin-induced alteration of membrane function we evaluated the frequency of aggregation sites in each bladder studied. For this, a single random micrograph was taken of each of at least ten different cells from each bladder, and the number of aggregation sites per 220 μm^2 of membrane was evaluated at × 45,000. After the quantitation was completed the identities of the

Table 1. Data for vasopressin-stimulated osmotic water flow and frequency of intramembranous particle aggregation sites; R-C, reference-control; Exp, experimental.

Hemi-bladder identity	Vasopressin concentration in serosal bath (milliunit/ml)	Vasopressin-stimulated osmotic water flow		Frequency of aggregation sites	
		Actual*	Relative† (%)	Actual‡	Relative† (%)
R-C A1	20	121	100	219	100
Exp A2	0	0	0	0	0
R-C B1	20	66	100	93	100
Exp B2	0.01	11	17	9	10
R-C C1	20	100	100	156	100
Exp C2	0.1	28	28	53	34
R-C D1	20	107	100	156	100
Exp D2	0.1	50	47	120	77
R-C E1	20	91	100	173	100
Exp E2	1	59	65	94	54
R-C F1	20	106	100	125	100
Exp F2	1	72	68	83	66
R-C G1	20	91	100	220	100
Exp G2	20	90	99	199	90

*Milligrams/(30 minutes × square centimeter). †Calculated for paired hemibladders. ‡Number per 220 μm^2 of membrane.

bladders were decoded and the frequency of aggregation sites was compared to corresponding osmotic water flow measurements (Table 1). There is clearly a linear relationship between these variables. Figure 2 illustrates the relationship ($r = .91$; $P < .005$) between relative osmotic water flow and the relative frequency of aggregation sites. A similar relationship ($r = .88$; $P < .005$) is also found between absolute osmotic water flow and absolute frequency of aggregation sites.

This finding suggests the possibility that the ordered aggregations of intramembranous particles observed in luminal membrane of granular cells after vasopressin stimulation might represent sites for vasopressin-induced osmotic water flow. Indeed, Pinto daSilva (*11*) has shown that intramembranous particles of red cell membranes may provide preferential structural pathways for water passage. However, since vasopressin is known to enhance the movement of Na^+, urea, and other solutes across the toad bladder (*12*), it should not be concluded that the rate of osmotic water flow is necessarily a specific function of particle aggregation frequency. Clearly, additional study is necessary to establish the functional significance of vasopressin-induced intramembranous particle aggregation. This report indicates that the distribution of intramembranous particles of granular cell luminal membranes is altered in vasopressin-stimulated toad bladder and suggests that this alteration may underlie vasopressin-induced changes in transport function.

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Speech Perception by the Chinchilla: Voiced-Voiceless Distinction in Alveolar Plosive Consonants

Abstract. *Four chinchillas were trained to respond differently to /t/ and /d/ consonant-vowel syllables produced by four talkers in three vowel contexts. This training generalized to novel instances, including synthetically produced /da/ and /ta/ (voice-onset times of 0 and +80 milliseconds, respectively). In a second experiment, synthetic stimuli with voice-onset times between 0 and +80 milliseconds were presented for identification. The form of the labeling functions and the "phonetic boundaries" for chinchillas and English-speaking adults were similar.*

Neither speech analysis nor speech synthesis techniques have led to a successful account of our perception of speech sounds in terms of invariant acoustic properties (*1*). For this reason and others (*1*), current theorists have hypothesized that at least some classes of speech sounds are recognized by "special processing" (*2, 3*). Speculation as to the nature of this special-to-speech processing varies: some believe that it involves "phonetic feature detectors" that presumably respond to rather complicated and abstract characteristics of the acoustic signal (*4*); others have espoused a "motor theory" of speech perception (*2, 3*), which suggests that one's tacit knowledge of the acoustic results of articulatory maneuvers somehow mediates the perception of speech. While variously described, these current theories suggest that speech perception is a species-specific behavior, and thus, in large part, a uniquely human ability. As Liberman stated, "Unfortunately, nothing is known about the way non-human animals perceive speech . . . however, we should suppose that, lacking the speech-sound decoder, animals would not perceive speech as we do, even at the phonetic level" (*2*).

We asked whether the chinchilla, a mammal with auditory capabilities fairly similar to man's (*5*), but certainly without a phylogenetic history of "phonetic knowledge," either acoustic or articulatory, could correctly classify a large number of naturally produced syllables on the basis of the voicing contrast. In experiment 1, we trained four chinchillas, using an avoidance conditioning procedure, to respond differently to a variety of spoken /t/ and /d/ consonant-vowel (CV) syllables. Once trained, these animals correctly classified novel instances of /t/ and /d/ syllables, including syllables produced by new talkers, those produced in new vowel contexts, and computer-synthesized /ta/ and /da/ syllables. In experiment 2, we presented synthetic stimuli that varied systematically from /da/ to /ta/ to the animals trained on natural speech, to animals not trained on natural speech, and to English-speaking adults for identification. The labeling functions and the "phonetic boundaries" were similar for all animal and human subjects.

The voicing feature, which distinguishes voiced (/bdg/) from voiceless (/ptk/) plosives in English, is appropriate for investigations of speech perception by animals since this distinction has been examined in cross-language studies of adults (*6, 7*) and infants (*8, 9*). The acoustic properties that distinguish voiced and voiceless plosives in absolute-initial, prevocalic, stressed position can be most readily described as a timing difference between the onset of the plosive burst and the onset of voicing (*6*), termed the voice-onset time (VOT). In synthetic speech, VOT can be varied along a continuum to produce plosives in which voicing precedes the plosive burst (prevoiced), begins nearly simultaneously with the burst (unaspirated), or lags behind the plosive burst (aspirated). The VOT is specified in milliseconds; negative values indicate that voicing leads and positive values indicate that voicing lags. In English, prevoiced and unaspirated plosives constitute the voiced phonemic category and aspirated plosives constitute the voiceless phonemic category. When English speakers identify synthetic tokens from the VOT continuum, perception changes abruptly from voiced to voiceless sounds; the VOT at which responses divide equally between voiced and voiceless is termed the "phonetic boundary." These boundaries differ with the place of articulation of the voiced-voiceless pair; the boundaries for labials