

Put together our longitudinal and training results provide strong support for the hypothesis that the awareness of rhyme and alliteration which children acquire before they go to school, possibly as a result of their experiences at home, has a powerful influence on their eventual success in learning to read and to spell. Although others have suggested a link between phonological awareness and reading<sup>3-5</sup> our study is the first adequate empirical evidence that the link is causal. Our results also show how specific experiences which a child has before he goes to school may affect his progress once he gets there.

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## Chloride impermeability in cystic fibrosis

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**Cystic fibrosis is the most common fatal genetic disease affecting caucasians and is perhaps best characterized as an exocrinopathy involving a disturbance in fluid and electrolyte transport<sup>1</sup>. A high NaCl concentration in the sweat is characteristic of patients with this disease; the basic physiological reason for this abnormality is unknown. We have microperfused isolated sweat ducts from control subjects and cystic fibrosis patients, and report here results which suggest that abnormally low Cl<sup>-</sup> permeability in cystic fibrosis leads to poor reabsorption of NaCl in the sweat duct, and hence to a high concentration of NaCl in the sweat.**

Sweat glands were microdissected from skin biopsies taken from five control subjects and three cystic fibrosis patients. All subjects were males between 23 and 28 yr old. Single sweat glands were dissected further to isolate the reabsorptive duct from the secretory coil. One end of the reabsorptive duct was then cannulated with a micropipette<sup>2</sup> which also served as a microelectrode in the lumen of the duct. To avoid significant changes in the composition of the perfusion fluids due to reabsorptive activity, duct lengths of 1.5-2.0 mm were perfused at rates > 100 nl min<sup>-1</sup> at 25 °C. The bathing solution always contained 150 mM NaCl while the duct was perfused with solutions of different composition (see Table 1). Transepithelial potential differences during perfusions were recorded via saturated KCl bridges connecting the bathing solution and the perfusion micropipette to a high impedance electrometer (Kiethley 610C) using Ag-Ag/Cl electrodes. Asymmetries without the duct in place were usually < ± 1.0 mV and were subtracted from all measurements.

During periods of perfusion in which there were identical concentrations (150 mM) of NaCl in the bath and in the lumen, the average spontaneous potential in control ducts was -6.8 mV, with the lumen negative with respect to the bath, while in the same conditions the average potential in ducts from cystic fibrosis patients was -76.9. This markedly increased negative potential in the cystic fibrosis duct cannot be due to increased Na<sup>+</sup> transport as suggested for cystic fibrosis respira-

**Table 1** Average luminal potential differences in cystic fibrosis and normal sweat ducts perfused with solutions of varying composition

	150 mM NaCl	50 mM NaCl	75 mM Na <sub>2</sub> SO <sub>4</sub>
Pre-ouabain control (n = 7)	-6.8 ± 2.5	-24.2 ± 2.4	-75.5 ± 11.1
CF (n = 5)	-76.9 ± 13.2*	-47.6 ± 12.3†	-33.3 ± 19.5
Post-ouabain control (n = 7)	-0.9 ± 0.5	-14.8 ± 1.3	-42.3 ± 6.5
CF (n = 5)	-0.9 ± 1.7	+9.9 ± 3.8*	-4.4 ± 1.7†

Values shown are the average luminal potential differences in sweat ducts microperfused with solutions of different composition before and after exposing the contraluminal surface of the duct to 5 × 10<sup>-6</sup> M ouabain. In addition to the principal solute indicated, all solutions contained: 2.5 mM KCl, 2.5 mM NaH<sub>2</sub>PO<sub>4</sub>, 1.0 mM CaCl<sub>2</sub>, 1.0 mM MgSO<sub>4</sub> and 10.0 mM glucose. Sufficient mannitol was added to hypotonic solutions to make all solutions iso-osmotic with plasma. More than one sweat duct from two of the control and two of the cystic (CF) subjects were microperfused so that the results represent the mean ± s.e.m. of seven control sweat ducts and five cystic fibrosis sweat ducts. Significant differences were tested by Student's *t*-test.

\* *P* < 0.001; † *P* < 0.05.

tory tissues<sup>3</sup>, as ductal reabsorption of Na<sup>+</sup> is only about one-third to one-fifth of that in normal glands<sup>4,5</sup>. It therefore seems likely that the elevated potential is due to a separation of charge brought about by a difference in the permeability of anions in the two tissues. To test this possibility, a 1:3 dilution diffusion potential for NaCl (dilute solution in the lumen) and a bi-ionic diffusion potential for Na<sub>2</sub>SO<sub>4</sub> (lumen) and NaCl (bath) were measured before and after inhibiting electrolyte transport with ouabain. In control ducts, the potential in the lumen before ouabain rose to -24.2 mV when the perfusate was 50 mM NaCl, indicating that Cl<sup>-</sup> is more permeable than Na<sup>+</sup> in control ducts. Changing the perfusate to Na<sub>2</sub>SO<sub>4</sub> in which the SO<sub>4</sub> anion is assumed to be relatively impermeable, caused the potential to increase further to -75.5 mV, which is almost identical to the potential observed in cystic fibrosis ducts perfused with 150 mM NaCl (Table 1). This result argues strongly that the mechanisms for active Na<sup>+</sup> reabsorption in the cystic fibrosis ducts are not impaired and that Cl<sup>-</sup> passively follows Na<sup>+</sup> out of the normal duct. In the cystic fibrosis duct, the luminal potential fell to -47.6 mV with 50 mM NaCl as the perfusate and to -33.3 mV with Na<sub>2</sub>SO<sub>4</sub> as the perfusate (Table 1). The drop in the potential with lower concentrations of NaCl in the lumen may be due to some extent to the diffusion potential contribution, but the reason for the decrease during perfusion with Na<sub>2</sub>SO<sub>4</sub> is not clear. As the potential in one duct did not fall during this perfusion, the decrease may be related to a variable, time-related reduction in tissue activity *in vitro*.

Treating the serosal side of the tissue with ouabain virtually abolished the spontaneous potential difference (Table 1). Nonetheless, the average dilution diffusion potential in control ducts was -14.8 mV, but in cystic fibrosis ducts the polarity of the potential was reversed with an average value of +9.9 mV. The change in sign for cystic fibrosis ducts in the absence of active transport, clearly demonstrates that the passive permeability properties of the epithelium are significantly different and reversed compared with normal ducts. Using the 'constant-field equation' (refs 6, 7) to solve for the ratio of the Na<sup>+</sup> permeability to the Cl<sup>-</sup> permeability ( $P_{Na}/P_{Cl}$ ) from the potentials generated in each tissue, we found that  $P_{Na}/P_{Cl}$  for controls was 0.26 while for cystic fibrosis patients it was 2.3. Thus the Cl<sup>-</sup> permeability in control ducts is almost an order of magnitude higher than in cystic fibrosis ducts. This calculated difference may be significantly underestimated as measured potentials may be less

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than actual potentials due to the possibility of tissue trauma. Calculations using the maximum potentials measured, rather than the average showed that  $\text{Cl}^-$  permeability in control ducts may be more than 40 times that in cystic fibrosis ducts.

Although we have not yet measured absolute permeabilities of  $\text{Cl}^-$ , the small potentials generated during perfusion with  $\text{Na}_2\text{SO}_4$  after ouabain treatment show that  $\text{Cl}^-$  permeability in the cystic fibrosis duct must be very low. The average bi-ionic potential generated across control ducts after ouabain was  $-42.3$  mV, indicating that  $\text{Cl}^-$  is significantly more permeable than  $\text{SO}_4$ . In contrast, this potential in cystic fibrosis ducts was only  $-4.4$  mV, indicating that  $\text{Cl}^-$  is only slightly more permeable than  $\text{SO}_4$ . As  $\text{SO}_4$  is generally considered to be almost impermeable across epithelia, the relatively low bi-ionic potential with  $\text{Cl}^-$  in cystic fibrosis ducts strongly suggests that  $\text{Cl}^-$  also has a very low permeability in this genetic variant of the tissue.

We believe that these data provide strong evidence that abnormally low  $\text{Cl}^-$  permeability is the basis of poor reabsorption of NaCl in the cystic fibrosis sweat duct and the correspond-

ing high concentration of NaCl in the sweat of these patients. Previously, it was reported that the potential difference across the respiratory epithelium in cystic fibrosis patients was dramatically increased compared with controls<sup>3</sup>, which was interpreted as being due to increased rates of  $\text{Na}^+$  reabsorption in the airways. In view of our findings, and as this is a genetic disease which should be expressed as a common defect in affected tissues, we believe that the observed potentials in the airways are more likely to be due to an abnormally low  $\text{Cl}^-$  permeability than to increased  $\text{Na}^+$  transport. If this is the case, it seems possible that a generalized defect in the  $\text{Cl}^-$  permeability may be closely associated with the fundamental disturbance in this disease and may further elucidate the problems associated with characteristic abnormalities of the pancreas, intestine and lung.

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## $T^{\text{Or}1}$ is a novel, variant form of mouse chromosome 17 with a deletion in a partial $t$ haplotype

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Moutier<sup>1</sup> discovered, in a mouse from a noninbred Swiss/Orleans laboratory stock, a spontaneous dominant mutation which mapped to the  $T$  locus, and which was named  $T^{\text{Or}1}$ . Genetic analyses indicated that  $T^{\text{Or}1}$  was not a simple mutation at one locus, but rather a deletion over a 3-centimorgan region of chromosome 17 that included both  $T$  and *quaking* ( $qk$ )<sup>2,3</sup>. Further experiments reported by Erickson *et al.*<sup>3</sup>, and a more comprehensive study by Hammerberg<sup>4</sup>, have demonstrated that  $T^{\text{Or}1}$  is associated with recessive genetic properties affecting sperm function, characteristic of the proximal region of complete  $t$  haplotypes. These results were interpreted as evidence for the location of proximal  $t$  haplotype 'sperm factors' within the region deleted by  $T^{\text{Or}1}$ . We now provide conclusive biochemical and genetic evidence that the ' $T^{\text{Or}1}$  haplotype' is inseparably associated with a chromosomal region derived from a naturally occurring mouse  $t$  haplotype. Hence, it is likely that the  $t$  haplotype properties of  $T^{\text{Or}1}$  are a consequence not of the deletion itself, but of closely linked mutant  $t$  haplotype genes.

The dominant mutation *Brachyury* ( $T$ ) on a mouse chromosome 17 causes a reduction in the tail length of  $T/+$  heterozygotes, and embryonic lethality in  $T/T$  homozygotes<sup>5</sup>. The  $T$  mutation also interacts with naturally occurring, recessive  $t$  haplotypes to cause taillessness in compound  $T/t$  heterozygotes. This last property of  $T$  has been used to identify cryptic  $t$  haplotypes carried by animals from many wild mouse populations. The new mutation  $T^{\text{Or}1}$  was found to exhibit all of the classical phenotypic properties of the original  $T$  mutation:  $T^{\text{Or}1}/+$  heterozygotes have a short tail; both  $T^{\text{Or}1}/T$  and  $T^{\text{Or}1}/T^{\text{Or}1}$  embryos die *in utero*<sup>3,6</sup>; and  $T^{\text{Or}1}/t$  compound heterozygotes are tailless. In addition, the  $T^{\text{Or}1}$  mutation acts as a recessive mutant allele at the  $qk$  locus—all  $T^{\text{Or}1}/qk$  animals

express the recessive quaking phenotype of  $qk$  homozygotes<sup>2,3</sup>, and  $T^{\text{Or}1}/qk$  males also express the sterility phenotype characteristic of  $qk/qk$  males<sup>3</sup>. The accumulated data indicate that the  $T^{\text{Or}1}$  mutation is actually a deletion spanning at least the 3-centimorgan region of DNA between the loci of  $T$  and  $qk$  on chromosome 17.

The *Tcp-1* gene is located within the  $t$  complex and encodes a major testicular cell protein (TCP) called p63/6.9 (ref. 7). All naturally occurring  $t$  haplotypes carry the allele *Tcp-1*<sup>a</sup>, which encodes a variant protein p63/6.9a. With one exception, all of the hundreds of examples of chromosome 17 not obviously carrying a  $t$  haplotype analysed to date from inbred and feral mice express a different form of the protein, p63/6.9b (ref. 8). The single exception is that of  $T^{\text{Or}1}$ , which does express p63/6.9a (ref. 9), suggesting that the  $T^{\text{Or}1}$  mutation is, indeed, associated with a short segment of  $t$  haplotype DNA<sup>10</sup>.

A higher resolution, comparative two-dimensional gel analysis of TCPs synthesized by inbred mice congenic for a series

**Table 1** Transmission ratios of males carrying the  $T^{\text{Or}1}$  haplotype in *trans* to a distal  $t$  haplotype

Male	Distal $t$ haplotype	Offspring			$\chi^2$
		$T^{\text{Or}1}$	$t^{\text{distal}}$	% $t^{\text{distal}}$	
1715	$t^{\text{h}17}$	1	7	88	4.50
2083	$t^{\text{h}17}$	2	21	91	15.69
2130	$t^{\text{h}17}$	0	29	100	29.00
2701	$t^{\text{h}17}$	1	10	91	7.36
3167	$t^{\text{h}17}$	1	19	95	16.20
2568	$t^{\text{Lub}2}$	2	50	96	44.30
2708	$t^{\text{Lub}2}$	2	24	92	18.61
Total	$t^{\text{h}17}$ and $t^{\text{Lub}2}$	9	160	95	134.92

The matings shown schematically in Fig. 3 were set up to obtain the genotypes used in this experiment:  $T^{\text{Or}1}/t^{\text{h}17}$  and  $T^{\text{Or}1}/t^{\text{Lub}2}$  (see also Fig. 1 for a diagrammatic representation of these genotypes). Each male was mated with outbred non- $t$  haplotype ( $+/+$ ) females. Progeny that receive the  $T^{\text{Or}1}$  chromosome have a short tail, whilst progeny that receive the  $t^{\text{h}17}$  or  $t^{\text{Lub}2}$  chromosomes have a normal tail. These phenotypes were scored at birth.  $\chi^2$  values were calculated in comparison with the expected mendelian values.