Pathophysiology of Gene-Targeted Mouse Models for Cystic Fibrosis

BARBARA R. GRUBB AND RICHARD C. BOUCHER

Cystic Fibrosis/Pulmonary Research and Treatment Center, School of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

I. INTRODUCTION

Cystic fibrosis (CF) is a fatal genetic disease that reflects abnormal ion transport across a number of epithelial tissues. Most of the morbidity and mortality in the human CF patient is a result of pulmonary complications; however, gastrointestinal complications of the disease are usually the first to be noted in the neonate (13). Although it is now well established that the primary epithelial transport defect is a defect in cAMP-mediated Cl⁻ conductance (see below), Knowles et al. (80) initially detected an elevated rate of Na⁺ absorption across CF airway epithelia, which is now a hallmark of the human disease (80, 81, 84). Soon thereafter, it was noted that a defect in Cl⁻ permeability was also present in airway epithelia (81, 84), which was congruent with data from the sweat duct (101, 102). Although CF has been described in the literature for more than 40 years (13), cloning of the CF gene was accomplished just 8 years after the epithelial ion transport defects were identified.

Positional cloning of the CF gene was accomplished by an elegant series of experiments involving saturation...
mapping and chromosome walking and jumping techniques (79, 105, 106). The CF gene product, termed the cystic fibrosis transmembrane conductance regulator (CFTR), was shown to correct defective Cl\(^-\) conductance in cultured CF epithelial cells (41, 104). Ultimately, expression of CFTR in heterologous cells demonstrated that the CFTR protein functions in part as a cAMP-regulated Cl\(^-\) channel (2, 8, 11, 76). It is now widely accepted that the CFTR Cl\(^-\) channel is the predominant cAMP-regulated Cl\(^-\) channel in the apical membrane of epithelial cells and that genetic defects in the activity of this channel are the underlying cause of cystic fibrosis. More recently, it has been shown that CFTR also functions as a regulator of other ion channels, principally the epithelial Na\(^+\) channel (115).

Once the CFTR gene was cloned, the stage was set for the generation of an animal model of the disease. An animal model for CF would benefit the study of the disease in a number of ways. First, a more in-depth study of the pathophysiology of the disease could be undertaken in an animal model than is possible in humans. Second, in the human CF population, there is a marked heterogeneity of phenotypic expression of the disease in patients possessing identical genotypes. A CF animal model would allow the identification of genes that modify the severity of the CF phenotype as well as identification of environmental influences on disease severity. Third, an animal model would be useful for testing pharmacological strategies to modify disease severity. Finally, an animal model for CF would be useful for testing various gene therapy protocols to determine vector efficacy in correcting CF ion transport defects and measuring the duration of time that a correction can be maintained.

Just 3 years after the CFTR gene was cloned, the generation of the first CF mouse model was reported (114). Several other models followed shortly thereafter (39, 92, 103). To date, 10 CF mouse models have been described in the literature. This paper reviews the phenotype as well as pathophysiological, pharmacotherapeutic, and gene therapeutic studies reported in these models.

### II. CYSTIC FIBROSIS MOUSE MODELS

All of the CF mouse models have been generated by the same general technique. Once the CFTR gene is mutated in the desired fashion, this mutated gene is cloned into a targeting vector and inserted into murine embryonic stem cells, a pluripotent cell type capable of generating any murine cell. Homologous recombination occurs in a small percentage of the stem cells, with the mutated gene integrating into the homologous gene locus of the stem cell (85). The pool of stem cells is then screened to identify those cells into which the gene has correctly targeted. These stem cells are then isolated, expanded, injected into murine blastocyes, and transferred to a pseudo-pregnant foster mother. The embryo matures and produces a chimeric mouse, which is a blend of both the normal cells and the cells containing the targeted CFTR gene. These chimeric mice can be identified by coat color; if stem cells containing the mutant gene are from a mouse line characterized by a light coat color and the embryonic cells are from a dark murine strain, the resulting mouse will be characterized by a variegated coat color. The chimeric mice are then bred together, and if the injected targeted cells populate the germ cells (which happens by chance), the chimeras will transmit the targeted gene in a ratio of 1:2:1 (homozygous normal, heterozygous, homozygous mutant).

With the use of these basic techniques, six knockout mouse models have been generated to date (see Table 1). They differ in the region of the gene targeted and the method by which targeting was accomplished (see references in Table 1 for a complete description of the molecular techniques). With the exception of the cfr\(^{min1Hg}\) knockout mouse, the phenotype among the various knockout mouse models is fairly similar (see sect. II A). However, in the cfr\(^{min1Hg}\) CF mouse, because of the targeting strategy used in its generation (insertional rather than replacement gene targeting), exon skipping and aberrant splicing produce some normal CFTR mRNA (40), resulting in a much milder gastrointestinal phenotype than exhibited by the other knockout mouse models (see sect. II A).

In general, most CFTR mutations result in loss of function due to abnormal processing of CFTR and failure to insert CFTR in plasma membranes. Therefore, gene-targeting strategies leading to absence of CFTR production may be expected to produce animals that mimic these forms of CF. This reasoning thus led to gene-targeting strategies focused on disrupting exons 10, 1, etc. However, the importance of creating a ΔF508 CF mouse model

### Table 1. Gene-targeted mouse models of cystic fibrosis

<table>
<thead>
<tr>
<th>CF Mouse</th>
<th>Molecular Technique (Reference No.)</th>
</tr>
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<tbody>
<tr>
<td>cfr(^{min1Hg})</td>
<td>Inframe stop (exon 10) (114)</td>
</tr>
<tr>
<td>cfr(^{min1Hg})</td>
<td>Insertion (exon 10) (39)</td>
</tr>
<tr>
<td>cfr(^{min1Hg})</td>
<td>Insertion (exon 10) (103)</td>
</tr>
<tr>
<td>cfr(^{min1Hg})</td>
<td>Duplication (exon 3) (92)</td>
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<td>Inframe stop (exon 2) (70)</td>
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<td>cfr(^{min1Hg})</td>
<td>Insertion (exon 1) (107)</td>
</tr>
<tr>
<td>cfr(^{min1Hg})</td>
<td>Homologous recombination (ΔF508) (131)</td>
</tr>
<tr>
<td>cfr(^{min1Hg})</td>
<td>Homologous recombination (ΔF508) (119)</td>
</tr>
<tr>
<td>cfr(^{min1Hg})</td>
<td>Homologous recombination (ΔF508) (25)</td>
</tr>
<tr>
<td>cfr(^{min1Hg})</td>
<td>Homologous recombination (G551D) (36)</td>
</tr>
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Mouse names are in accordance with rules set forth by the International Committee on Genetic Nomenclature for Mice. CF, cystic fibrosis.
stems from the fact that this mutation accounts for >70% of the CFTR mutations in the human population (a 3-bp deletion of phenylalanine at position 508) (79, 105) and that specific processing abnormalities resulting from the ΔF508 mutation may be amenable to novel therapies. Because the murine CFTR gene is 78% homologous to its human counterpart and ΔF508 occurs in the same position in the murine gene (116), chances were good that deletion of this amino acid would produce a CF mouse model with certain similarities to the human ΔF508 mutation. Fortunately, ΔF508 CF mouse models exhibit the CFTR processing defect characteristic of the human mutation (see sect. vi).

The G551D CF (cftr<sup>TgHmlG551D</sup>) mouse is another recently generated mouse model (36). In the human population, this mutation is relatively common, and a genotype/phenotype relationship has been identified in that the incidence of meconium ileus is reduced threefold in patients with this mutation compared with those homozygous for ΔF508 (65). In the human, the G551D CFTR protein is processed normally, but the cAMP-regulated Cl<sup>-</sup> channel activity of G551D is much reduced (121). Indeed, the G551D CF mouse model appears to exhibit a reduction in neonatal gastrointestinal pathophysiology compared with that observed in other knockout mouse models (36) (see sect. IIIA).

III. ORGAN PATHOPHYSIOLOGY

A. Intestine

1. Histopathology

In the human subject, the intestinal manifestation of CF in the small intestine is characterized by decreased Cl<sup>-</sup> and fluid secretion, which may contribute to the meconium ileus (MI) in ~10% of CF newborns and intestinal obstruction and accumulation of mucus in older CF patients (43). Virtually 100% of CF infants with MI are pancreatic insufficient, and it has been suggested that lack of pancreatic enzyme digestion of the meconium may contribute to MI (14). Analysis of the meconium from CF infants reveals that it contains less water and is more viscous than normal stool (112), accounting for the intestinal obstruction typically observed within days of birth. Although GI complications occur less frequently in older patients, ~3% of adult CF patients still suffer from recurrent distal intestinal obstructions (96). Intestinal obstructions may be related to a combination of factors, including maldigestion, viscous mucus, and enhanced absorption of water and electrolytes. However, it is likely that the abnormality primarily reflects a decrease in the Cl<sup>-</sup> permeability of the apical epithelial membrane (84, 101).

An intestinal phenotype appears to be the hallmark of all CF mouse models. With the exception of two of these models (cftr<sub>tm1HGU</sub>, cftr<sub>tm1Eur</sub>) (39, 119), the intestinal pathology is fairly severe. Most of the models exhibit mucus accumulation in the crypts of Lieberkühn, goblet cell hyper trophy (42, 119), hyperplasia (42, 92, 107), and eosinophilic concretions in the crypts (36, 70, 92, 114). Although the pathological changes have been described as occurring throughout the intestinal tract, studies on the cftr<sub>tm1Unc</sub> mouse have reported milder pathology in the duodenum that increased in severity distally (although expression can be variable) (42, 114). Indeed, the ileocecal (36, 92, 114, 131) and large intestinal regions (114) appear to be the most common sites of intestinal blockage and rupture in the most severely affected mouse models. Although occurring less frequently, jejunal obstructions have also been reported in several CF mouse models (103, 114).

So severe are the intestinal complications that a large fraction (50–95%) of the CF mouse pups die before the age of weaning (Fig. 1A) (70, 103, 107, 114, 131). In most of the CF mice, the intestinal morbidity and mortality is manifest at two distinct time periods. The first subset of CF mice die within 5 days of birth, and the second subset dies shortly after weaning (103, 114). The high periwearing mortality appears to be because of consumption of solid food, since it has been found that placing the CF mice on a liquid diet (42, 78) greatly prolongs the life span of these animals (Fig. 1B). The liquid-fed CF mice gain weight at the same rate as normal littermates, although the former remained significantly smaller than the control mice (78). We have found that substituting drinking water with an electrolyte solution containing 6% polyethylene glycol (Colyte) (58), which allows consumption of mouse food, also greatly prolongs the life span of the cftr<sub>tm1Unc</sub> CF mouse, with survival curves similar to those in Figure 1B. The Colyte solution has advantages over the liquid diet in that it is much less expensive, and daily drinking bottle changes and sterilizations are no longer necessary.

The two mouse models (cftr<sub>tm1HGU</sub>, cftr<sub>tm1Eur</sub>) that exhibit relatively little intestinal pathology also appear to experience no increase in death due to gastrointestinal complications compared with normal mice (39, 119). The cftr<sup>TgHmlG551D</sup> mouse model also exhibits a significantly greater rate of postweaning survival (~70%) than seen in most other mouse models (36). Possible reasons for these increased survival rates are discussed below.

2. Physiology

A) cAMP-MEDIATED CHLORIDE SECRETION. With respect to intestinal physiology, all of the CF mouse models have a very similar physiological phenotype to that of the human CF intestine, i.e., defective cAMP-mediated Cl<sup>-</sup> conductance. All of the mouse models exhibit a significant decrease in the basal electrical potential difference (PD) and short-circuit current (I<sub>sc</sub>) (a measure of active ion
The expression of wild-type functional CFTR undoubtedly explains this observation. In agreement with the expression and function of CFTR, these mice exhibit no significant morbidity or mortality due to intestinal complications. The cftrtm1Eur (ΔF508) mouse also exhibits no reduction in life span due to intestinal complications and, likewise, shows a rather substantial Cl⁻ secretory response to an increase in cellular cAMP (119). This ΔF508 mouse expresses levels of mRNA for ΔF508 CFTR that appear comparable to the levels of wild-type CFTR in control animals (49). In contrast, the other two ΔF508 mouse models (cftrtm1Kth, cftrtm2Cam) exhibit a marked reduction in mRNA levels for ΔF508 CFTR in intestinal epithelia (25, 131). The authors speculate that this high level of ΔF508 CFTR mRNA in the cftrtmlEur mouse may allow more of the mutant CFTR to be correctly processed, allowing more functional protein to reach the plasma membrane. Several studies have shown that the ΔF508 CFTR protein exhibits partial function as a Cl⁻ channel (49), with a similar single conductance and a decrease in open channel probability (30).

The cftrTgHm1G551D mouse exhibits a very small cAMP-mediated Cl⁻ secretory response (4–5% of normal) in both the small and large bowel (36). Although this mouse model has a significant mortality due to intestinal complications (33% die before 35 days of age), the incidence of death is lower than most other CF mouse models. Interestingly, in human CF infants homozygous for this mutation, transport; Fig. 2A), likely because of the decreased basal rate of Cl⁻ secretion. Without exception, all of the models also exhibit a significant decrease (36, 113, 119) or complete absence of cAMP-mediated Cl⁻ secretion (21, 25, 70, 103, 131) across the intestinal epithelium when preparations are studied in vitro. In the cftrtm1Unc mouse, all intestinal regions from the duodenum to the distal colon exhibited defective cAMP-regulated Cl⁻ transport (59) (Fig. 2B).

In the CF mouse models that do exhibit some degree of cAMP-mediated Cl⁻ secretion, this phenomenon appears to be positively correlated with the absence of gut disease. In the cftrtm1Hgu mouse created by an insertional mutation to disrupt exon 10, up to 10% wild-type CFTR mRNA is expressed in the airway epithelia and as much as 20% expression is detectable in the intestine (40). In these animals, the cAMP-mediated Cl⁻ secretory response in the jejunum was only reduced by ~50% compared with normal mice (113). The expression of wild-type functional CFTR undoubtedly explains this observation. In agreement with the expression and function of CFTR, these mice exhibit no significant morbidity or mortality due to intestinal complications. The cftrtm1Eur (ΔF508) mouse also exhibits no reduction in life span due to intestinal complications and, likewise, shows a rather substantial Cl⁻ secretory response to an increase in cellular cAMP (119). This ΔF508 mouse expresses levels of mRNA for ΔF508 CFTR that appear comparable to the levels of wild-type CFTR in control animals (49). In contrast, the other two ΔF508 mouse models (cftrtm1Kth, cftrtm2Cam) exhibit a marked reduction in mRNA levels for ΔF508 CFTR in intestinal epithelia (25, 131). The authors speculate that this high level of ΔF508 CFTR mRNA in the cftrtmlEur mouse may allow more of the mutant CFTR to be correctly processed, allowing more functional protein to reach the plasma membrane. Several studies have shown that the ΔF508 CFTR protein exhibits partial function as a Cl⁻ channel (49), with a similar single conductance and a decrease in open channel probability (30).

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**FIG. 1.** A: survival curve from homozygous normal and homozygous cystic fibrosis (CF; cftrtm1Unc) mouse pups. Mouse pups had access to normal mouse food and were weaned at day 21. [Adapted from Snouwaert et al. (114). In original figure, data for heterozygotes were also provided. These pups had a very slightly reduced survival compared with homozygous normal pups.] B: effect of liquid diet on survival of CF (cftrtm1Unc) mouse pups. Mice were weaned at day 21 to either solid food or liquid diet. [Adapted from Kent et al. (78).]

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**FIG. 2.** Basal short-circuit current (Iₑ) (A) and forskolin-stimulated Iₑ increase (B) in 4 regions of normal (open bars) and CF (solid bars) (cftrtm1Unc) murine intestine. Data shown are means ± SE; n = 3 for duodenums (Duod), and n = 7 for other regions (Jej, jejunum; colon, proximal colon). *P < 0.01 vs. normal preparations. [From Grubb (59). Reprinted with permission from Elsevier Science.]
there is a threefold decrease in the incidence of MI compared with homozygous $\Delta F508$ infants (65). In contrast to the $\Delta F508$ CFTR protein, the G551D CFTR protein appears to be normally processed in humans, but it exhibits a markedly reduced cAMP-mediated Cl$^-$ conductance (121). Thus it would appear that although sufficient amounts of the G551D protein are produced and correctly processed to the apical membrane, the relatively low levels of Cl$^-$ channel function are not sufficient to protect either the human or murine intestine from the classical CF complications.

**B) CALCIUM-MEDIATED CHLORIDE SECRETION.** Although the normal human intestine reacts to agonists that increase intracellular Ca$^{2+} -$(ionomycin, carbachol, bethanecol) with a Cl$^-$ secretory response, the intestinal tract of human CF patients is unresponsive to these agents (12, 91, 117). The CF mouse intestine appears to be remarkably similar to that of its human homolog with respect to Ca$^{2+}$-activated Cl$^-$ secretory responses. The normal murine intestine reacts to agonists that increase intracellular Ca$^{2+}$ with a Cl$^-$ secretory response, whereas animals lacking functional CFTR exhibit no Cl$^-$ secretory response to these agents (22, 29, 59, 70) (Fig. 3). Again, the cftr$^{tm1Hgu}$ mouse was found to exhibit a small but less than normal Cl$^-$ secretory response to carbachol (113), which is consistent with the presence of low levels of functional wild-type CFTR.

The absence of an intracellular Ca$^{2+}$-mediated Cl$^-$ secretory path in either CF human or murine intestinal epithelia contrasts directly with airway epithelium from each species. A combination of anion selectivity, blocker studies, and CF knockout mouse studies has led to the conclusion that airway epithelial cells have a molecularly unique intracellular Ca$^{2+}$-activated Cl$^-$ channel (Cl$_A$) (3, 15, 22, 35) (see sect. III B). In intestinal epithelial cells, however, it is now fairly certain that only one Cl$^-$ channel, the cAMP-activated CFTR, is expressed in the apical membrane. Although intestinal tissues from normal subjects respond to agonists that increase intracellular Ca$^{2+}$ with a Cl$^-$ secretory response, it is thought that this response is due to a Ca$^{2+}$-activated basolateral K$^+$ conductance, which induces cellular hyperpolarization and increases the driving force for Cl$^-$ secretion via CFTR (22, 31). Because the CFTR channel is defective in human CF patients, and absent in the CF mouse models described, this mechanism of “secondary” Cl$^-$ secretion induced by cell hyperpolarization is also defective in the CF intestine. It has been proposed that the failure of the CF mouse to exhibit airway disease is due to the prominence of Cl$_A$ (22) (see sect. III B). Because the intestinal epithelium is not thought to express an alternative Cl$^-$ secretory pathway, severe intestinal pathophysiology dominates the CF murine models. [However, there is some suggestion that certain mouse strains may express Cl$_A$ and consequently exhibit a less severe phenotype (see sect. IV).]

**C) SODIUM-GLUCOSE COTRANSPORT.** The small intestines of most mammalian species, including humans and mice, exhibit electrogenic Na$^+$ absorption linked to glucose uptake across the apical membrane. The transport protein responsible for the apical intestinal Na$^+$-glucose cotransport (SGLT1) has been cloned (72). In the CF human intestine, it has been reported by some that the rate of Na$^+$-glucose transport is upregulated (7, 48). However, a recent report, using brush-border membrane vesicles from human duodenum and jejunum, found that there was no difference in the Na$^+$-glucose transport rate in membrane vesicles harvested from the CF intestine compared with control (9). Likewise, several studies detected no upregulation in the rate of Na$^+$-glucose cotransport across the intestine of several of the CF mouse models (39, 58, 119) (Fig. 4). Serendipitously, the cftr$^{tm1Unc}$ mouse was useful in revealing the regulation of Na$^+$-glucose.

**FIG. 3.** Effect of bethanecol (10$^{-5}$ M) and ionomycin (5 $\mu$M) on $I_{sc}$ in normal (open bars) and CF (solid bars) (cftr$^{tm1Hgu}$) murine jejunum. Data shown are means ± SE. For bethanecol, $n = 7$ normal and $n = 6$ CF; for ionomycin, $n = 11$ normal and $n = 6$ CF. * $P \leq 0.01$ vs. normal preparations. [From Grubb (59). Reprinted with permission from Elsevier Science.]

**FIG. 4.** Effect of apical glucose on $I_{sc}$ in normal (□; $n = 8$ at each point) and CF (●; $n = 10$ at each point) murine jejunum (cftr$^{tm1Unc}$). [From Grubb (58).]
transport rates across the jejunum by cellular cAMP, albeit at comparable efficiencies in both normal and CF mice (58).

D) BICARBONATE SECRETION. Bicarbonate secretion is important, especially in the duodenum, to protect the intestinal mucosa against damage from the high levels of acid produced by the stomach. However, other regions of the intestinal tract are capable of HCO\textsubscript{3} secretion as well. Evidence suggests that the cftr\textsuperscript{tm1Unc} jejunum has a defect in the ability to secrete HCO\textsubscript{3}. In Ussing chamber studies of jejunal preparations, ion substitution experiments revealed that the basal I\textsubscript{sc} in normal jejunum primarily reflects Cl\textsuperscript{−} secretion but contains a component of HCO\textsubscript{3} secretion as well (58). In contrast, in CF jejunum, neither Cl\textsuperscript{−} nor HCO\textsubscript{3} was spontaneously secreted, nor could secretion of these anions be induced via cAMP stimulation. Another study reported a defect in the ability to secrete HCO\textsubscript{3} in the duodenum of the cftr\textsuperscript{tm1Unc} mouse (69).

Bicarbonate transport by murine intestine also shows similarities to the human intestine. Normal human jejunum exhibits a small secretion of HCO\textsubscript{3} in response to theophylline, whereas CF jejenum fails to respond to this agent, suggesting a defect in the ability of the CF intestines to secrete HCO\textsubscript{3} in response to cAMP (118). Although the mechanism(s) in the defect of HCO\textsubscript{3} secretion in CF tissue is unknown, there are reports in the literature suggesting that CFTR can conduct HCO\textsubscript{3} but at a reduced rate compared with Cl\textsuperscript{−} (46). Other possible candidates for transporting bicarbonate that may be impaired in CF have been discussed previously (58).

E) ELECTRONEUTrAL SODIum Chloride ABSORPTION. Electroneutral NaCl absorption is another major route of Na\textsuperscript{+} absorption across the mammalian small intestine. This transport is most likely due to coupling via a pair of parallel exchangers (Na\textsuperscript{+}/H\textsuperscript{+} and Cl\textsuperscript{−}/HCO\textsubscript{3}) (4).

In addition to stimulating Cl\textsuperscript{−} secretion in the normal small intestines, cAMP has also been shown to have an antiabsorptive effect due to inhibition of coupled NaCl absorption (45). Although little work has been done on this aspect of ion transport across CF intestinal epithelia, it has been reported that cAMP does not inhibit electroneutral Na\textsuperscript{+} absorption in human CF patients (12). Furthermore, another study reported that cAMP actually increased the rate of electroneutral NaCl entry into CF human intestinal epithelia (91). One study investigating this transport process in the murine jejunum showed that cAMP simultaneously inhibited net electroneutral NaCl absorption and induced electrogenic Cl\textsuperscript{−} secretion in normal intestinal epithelia, whereas in the cftr\textsuperscript{tm1Unc} CF intestine, cAMP failed to inhibit electroneutral NaCl absorption (23). The authors speculate that CFTR may be required (either directly or indirectly) for cAMP inhibition of electroneutral NaCl absorption by the small intestine. These data also suggest that CFTR may be localized and functional in both the crypts and villi of the small intestine.

It should be noted that others report that forskolin does downregulate fluid absorption in the ileum of the CF mouse (27, 34). Therefore, it is likely that the lack of response reported for the CF jejunum may be region specific.

F) AMILORIDE-SENSITIVE SODIUM ABSORPTION. The distal colonic epithelia of a number of species exhibit electrogenic Na\textsuperscript{+} absorption that is inhibited by the diuretic amiloride. In some species (rabbit and human), this type of Na\textsuperscript{+} absorption is evident when a normal diet is fed (111, 120). However, in both the rat and mouse, little amiloride-sensitive Na\textsuperscript{+} absorption is seen in the distal colon when the animal is on a normal diet (60, 64). [The magnitude of amiloride-sensitive Na\textsuperscript{+} absorption in the distal colon of mice on a normal diet can differ substantially between strains of mice (unpublished data)]. However, when aldosterone levels are stimulated by a low-Na\textsuperscript{+} diet (or aldosterone is given exogenously), electrogenic Na\textsuperscript{+} absorption manifests in these species (60, 64).

Electrogenic, amiloride-sensitive Na\textsuperscript{+} absorption is of interest to those studying Na\textsuperscript{+} transport in CF tissue because it is markedly upregulated in human CF airway epithelia (16, 80). This upregulation has been shown to be related to the lack of functional CFTR (115). There are reports in the literature suggesting an upregulation of electrogenic amiloride-sensitive Na\textsuperscript{+} absorption across the rectums and colons of CF patients (56, 94). However, other studies find no difference in amiloride-sensitive Na\textsuperscript{+} absorption across the CF human rectal epithelia compared with rates exhibited by normal tissue (55, 66).

Studies comparing electrogenic Na\textsuperscript{+} absorption across the colon of CF versus normal mice found no differences in Na\textsuperscript{+} transport between the genotypes when the mice were maintained on a normal diet (29, 39, 60). However, as pointed out above, amiloride-sensitive Na\textsuperscript{+} absorption in these mice on a normal diet ranged from zero to very low. When mice were placed on a low-Na\textsuperscript{+} diet to stimulate aldosterone production, the distal colonic epithelia of CF mice (cftr\textsuperscript{tm1Unc}) exhibited a significantly enhanced amiloride-sensitive Na\textsuperscript{+} absorption compared with controls (60). However, this finding was complicated by the fact that the CF mice also exhibited a significantly greater level of plasma aldosterone when placed on a low-Na\textsuperscript{+} diet compared with the normal animals. When mice of both genotypes were given a constant dose of aldosterone via osmotic pumps, the CF mice continued to exhibit a significantly enhanced amiloride-sensitive I\textsubscript{sc} compared with controls. These data support the hypothesis that CFTR exhibits a regulatory relationship with the Na\textsuperscript{+} channel and that the two channels must be located in close proximity in the apical membrane of the colonicocyte.

3. Transgenic correction of CF murine intestine

The intestinal histopathology and pathophysiology of the cftr\textsuperscript{tm1Unc} mouse have been partially corrected by
expression of human CFTR (cDNA) driven by an intestinal specific promoter, the rat intestinal fatty-acid binding promoter gene (133). Two founder lines expressing the transgene were studied; in these animals, human CFTR (hCFTR) mRNA was most abundant in the ileum, jejunum, and duodenum, with much less expression in the colon and cecum. However, unlike wild-type CFTR, the hCFTR mRNA was not expressed in the crypts, but rather in the villi. In contrast, wild-type CFTR mRNA is found in abundance in the colonic region, primarily localized to the crypts. In the gut-corrected transgenic mice, goblet cell hyperplasia was entirely corrected in the small intestine but not in the colon of the CF knockout mice. Furthermore, the jejuna exhibited a small but significant Cl⁻ secretory response to forskolin that was absent in the colon (133). Although hCFTR expression in these transgenic CF mice appears to be localized primarily to the villi of the small intestine, the Cl⁻ secretory capacity appears sufficient to prevent gut obstruction, and there was no increased mortality in these transgenic CF mice compared with normal animals. This strategy appears to be useful for increasing the longevity of the CF mice as well as providing information on the quantity and location of CFTR in the intestinal tract necessary for normal function. Other strategies, primarily dietary, have also been useful in prolonging the life span of the various CF mouse models without disrupting the pathophysiological manifestations in other organs (42, 58, 78).

4. Heterozygote advantage

Most of the CF mouse models generated to date closely mimic human CF gastrointestinal pathophysiology. This feature presented a unique opportunity to assess one of the most widely speculated questions regarding CF, that of a “heterozygote advantage.” A heterozygote advantage is most plausible to explain the maintenance of the high CF heterozygote frequency in the human population. Although several CF-selective advantages have been proposed, only resistance to secretory diarrhea (e.g., cholera) is supported by the knowledge that CFTR is a cAMP-regulatable Cl⁻ channel (8). The hypothesis is indirectly supported by previous reports that showed decreased sweat secretion in response to β-adrenergic stimulation of CF heterozygotes compared with normals (10, 108) and cholera stimulation of control but not CF human intestinal epithelium (6, 117). The availability of the mouse model for cystic fibrosis provided the first opportunity to directly test the hypothesis of CF heterozygote resistance to cholera. Analysis of CFTR protein expression levels, Cl⁻ secretion, and in vivo fluid accumulation in all three genotypes [normal CFTR(−/−), heterozygote CFTR(−/+), and CF CFTR(−/−)] from an isogenic strain of the cfrt<sup>tm1Unc</sup> mouse showed that there was a direct correlation in all three genotypes between CFTR expression and function in response to cholera toxin (52). The study suggests that the lower level of CFTR expression in CF heterozygotes translates into decreased fluid secretory responses to cholera and other bacterial secretagogues, constituting an effective protective mechanism to avoid the toxin-mediated dehydration that is often life-threatening. This protection against a fully developed toxin-mediated diarrhea provides a potential explanation for the high incidence of CF carriers, i.e., selection due to improved heterozygote survival in the face of toxigenic diarrheas.

Two other reports have also investigated this hypothesis, with variable results. In the first study, which used the same cfrt<sup>tm1Unc</sup> mouse although electing not to use an isogenic strain, a reduced Cl⁻ conductance in a significant number of CFTR(−/) mice compared with CFTR(+/+) mice was observed (23). The second study investigated homozygote normal and heterozygote Cl⁻ secretory responses in the colon of the CFTR(+/+) and the CFTR(−/−) cfrt<sup>tm1Com</sup> mouse (28). Although no difference in short-term acute stimulation of Cl⁻ secretion was detected, the authors did advance the important insight that prolonged stimulation [as was initially performed (52)] may reveal a heterozygote advantage. Importantly, both of these latter two studies did not utilize an isogenic strain of mice, and a recent study has suggested that modifier genes are present in different strains, which might mask differences between heterozygote and normal responses (124).

In summary, the intestinal pathophysiology of the CF mouse has proven to be remarkably similar to its human counterpart, exhibiting both defects of macromolecular secretion (mucus plugging) and ion transport (reduced or absent Cl⁻ and HCO₃⁻ secretion and enhanced Na⁺ absorption). The diversity of mouse models and strains on which the CFTR mutations are bred allows for extensive geno-type/phenotype studies and investigations of other factors modifying disease severity. Furthermore, as has already been demonstrated, these mice will undoubtedly be useful in elucidating basic ion transport physiology as well.

B. Airway Epithelium

The airways of CF mice are of obvious interest to investigators because ~95% of the morbidity and mortality in CF humans is due to pulmonary manifestations of the disease (see Ref. 32). In the CF patient, a consistent finding in the airways is mucus plugging with bacterial infection (13). As the disease progresses, bronchiolitis and bronchitis/bronchiectasis, goblet cell hyperplasia extending into the bronchioles, and submucosal gland hypertrophy are also classic findings of the disease (13).

Unlike the reports of severe gastrointestinal pathology in the first CF mouse models, a surprising lack of pulmonary pathophysiology was noted in these mice.
However, because most of the animals examined were quite young and raised in a semisterile barrier environment, it was hoped that as the mice matured and/or were removed from the barrier environment, airway pathology would manifest itself as it does in the CF human infant.

1. Histopathology

In the cftr<sup>tm1Unc</sup> CF mouse, the pathology reported for the airways was confined to the upper airways, and the findings were somewhat surprising, e.g., the CF mice exhibited marked atrophy of the serous gland tissue in the dorsolateral sinus (114). Others have also reported nasolacrimal gland distension in this CF mouse model (78). Also, there are reports in this CF mouse model that the submucosal glands (upper trachea) are distended (78), with dilation of the submucosal gland ducts but no acinar hyperplasia (114). In the G551D mouse model (36), approximately one-third of the CF animals exhibit inspissated eosinophilic material in the lumen of the pharyngeal submucosal glands.

In all CF mouse models examined, virtually normal lung histology and absence of mucus plugging are consistent findings (36, 39, 70, 78, 92, 103, 114, 119). The hypothesis that older CF mice raised in a less sterile environment may exhibit lung disease does not appear to have been substantiated. Cystic fibrosis mice (cftr<sup>tm1Unc</sup>) over 2 yr old and kept out of the barrier facility for over 1 yr have failed to exhibit lung disease (Grubb, unpublished data; B. Koller, personal communication). Others have noted that even upon reaching adulthood, CF mice (cftr<sup>tm1Hgu</sup>) did not exhibit pulmonary pathology (32). However, it has been reported that the cftr<sup>tm1Hgu</sup> CF mice when repeatedly exposed to nebulized Staphylococcus aureus over a long term (1–2 mo) exhibit a significantly greater incidence of goblet cell hyperplasia, mucus retention, and bronchiolitis than normal littermates (32). Also, these pathogen-exposed CF mice exhibited a significantly greater number of pulmonary colonies of <i>S. aureus</i> and <i>B. cepacia</i>, indicating a reduction in the ability to clear these opportunistic pulmonary pathogens (32). However, it should be stressed that none of the CF mouse models appears to experience an increase in pulmonary pathology under normal housing conditions. The reasons for the lack of similarity of the human and mouse model with respect to airways disease are discussed in section II.B4.

2. Physiology

In the human CF patient, both the upper (nasal) and lower (trachea, bronchi) airways exhibit hyperabsorption of Na<sup>+</sup> (80, 81, 84) and reduced or absent cAMP-mediated Cl<sup>−</sup> secretion (84, 122). The hyperabsorption of Na<sup>+</sup> and osmotically linked water absorption of the airway epithelium is thought to contribute to thick, sticky mucus, and possibly a reduction in the volume of airway surface liquid, thus decreasing mucociliary clearance and predisposing airways to disease. A reduction in CFTR function may be especially important in submucosal glands, where CFTR is found in relative abundance in the serous cells and ducts (44). Lack of Cl<sup>−</sup> secretion in the glands may change the composition of the mucus as well as impede the ability of mucus to be flushed from the glands.

3. Upper airways

In human subjects, the electrical potential (PD) across the nasal mucosa in vivo was first used to demonstrate hyperabsorption of Na<sup>+</sup> across the airway epithelium in CF patients (80) (Fig. 5A). The same technique has been applied to the mouse. In the various CF mouse models for which data are given (including the knockout, ΔF508, and G551D models), a consistent finding with respect to airways physiology is hyperabsorption of Na<sup>+</sup> across the nasal mucosa as indicated by a significantly lower basal PD compared to normal littermates.

![Fig. 5A: comparison of in vivo basal transnasal electrical potential difference (PD) in normal and CF (cftr<sup>tm1Unc</sup>) mouse and normal and CF human patients.](image-url)

![Fig. 5B: change in PD](image-url)
enhanced baseline nasal PD in vivo (36, 63, 107, 113, 119, 131) (Fig. 5A). All of these CF mice respond to amiloride, a drug that blocks electrogenic Na⁺ absorption, with a significantly greater decrease in the nasal PD than in control mice.

To estimate the relative Cl⁻ permeability of the apical membrane, a low Cl⁻ solution (either with or without an agent that increases intracellular cAMP) is perfused on the nasal mucosa. In normal mice (and humans), this results in a hyperpolarization of the transmucosal PD (Fig. 5B). In CF subjects, however, this maneuver results in no change in transmucosal PD or a slight depolarization of the basal PD (Fig. 5B). With the exception of two CF mouse models (cftr

\[ \text{F508} \] CF mouse exhibits a significantly reduced response to the drug protocol. The cftr

\[ \text{Eur} \] CF mouse exhibited a normal response to this protocol. (Interestingly, both of these mouse models exhibit almost no gut pathology; see sect. IIIA.) As previously mentioned, the cftr

\[ \text{Hgu} \] CF mouse exhibits ~10% wild-type CFTR mRNA in the lung (40), which likely explains the response to the low-

\[ \text{Cl}^- \] perfusion. The F508 CF mouse (cftr

\[ \text{Eur} \]) expresses levels of mRNA for the mutated CFTR that appear comparable to the levels of wild-type CFTR in control animals (49). The authors speculate that this high level of F508 CFTR mRNA may allow more of the mutant CFTR to be correctly processed and thereby allow more partially functional F508 protein to reach the plasma membrane. In contrast, at least in some tissues, the other two F508 models (25, 131) exhibit a marked reduction in mRNA levels for the mutated CFTR.

The conclusion that Na⁺ is hyperabsorbed across the CF mouse nasal mucosa, based on the raised basal PD in vivo, has been confirmed in freshly excised nasal mucosa mounted in small-diameter Ussing chambers. The freshly excised nasal mucosa from CF mice (cftr

\[ \text{Hgu} \]) exhibit a significantly enhanced basal L⁰ (63) compared with littermate controls (Fig. 6A). The amiloride-sensitive L⁰ is ~3.3 times greater in the CF epithelia and accounts for virtually all of the basal L⁰ in both the CF and normal nasal epithelia (Fig. 6A). Similar results were obtained for tissues bathed in bilateral Cl⁻-free Ringer solution. Therefore, these results cannot be explained by an amiloride-induced Cl⁻ secretory response in the normal tissue (see Ref. 63). These tissues were then treated with forskolin to increase the intracellular CAMP levels and induce Cl⁻ secretion. In CF nasal epithelia (Fig. 6B), the murine CF tissue exhibited virtually no response to forskolin, whereas the normal tissue responded with an increase in \( I_{sc} \), which has been shown to reflect Cl⁻ secretion. However, some CF murine nasal mucosa (cftr

\[ \text{Hgu} \]) exhibit a small Cl⁻ secretory response to forskolin (63). Because these CF mice express no CFTR protein, this Cl⁻ secretory response cannot be mediated via CFTR (see sect. III B4).

In human airway tissue, stimulated Cl⁻ secretion is mediated approximately equally by the CFTR channel and a molecularly distinct, alternative Ca²⁺-regulated channel (Cl₅₆) in the apical membrane (15). In human CF tissue, although the cAMP-stimulated CFTR pathway is defective, the Ca²⁺-mediated Cl⁻ secretory pathway is functional (15, 122) and has been reported by some to be upregulated in CF human airway epithelium tissue (75, 82). In the murine nasal mucosa, preparations from normal animals exhibit no response to ionomycin, a drug that increases intracellular Ca²⁺ (63) (Fig. 6B). In contrast, CF nasal...

![Fig. 6A](http://physrev.physiology.org/)

**Fig. 6.** A: basal \( I_{sc} \) of excised nasal epithelia studied on small-aperture Ussing chambers in vitro. Note that basal \( I_{sc} \) of epithelia from CF mice (cftr

\[ \text{Hgu} \]) is significantly elevated compared with normal. Also, amiloride-sensitive \( I_{sc} \) (an indication of Na⁺ absorption) is significantly elevated in CF tissue. B: forskolin (10⁻⁵ M) elicits a significant change in \( I_{sc} \) (a Cl⁻ secretory response, see Ref. 63) in normal nasal epithelia and virtually no response in CF tissue. Ionomycin (5 × 10⁻⁶ M) elicits a small decrease in \( I_{sc} \) of normal nasal epithelium and a significant increase in \( I_{sc} \) (representing Cl⁻ secretion) in CF tissue. All studies were done in presence of bilateral Krebs Ringer bicarbonate buffer. Data shown are means ± SE, with sample sizes shown in parentheses. Open bars are for normal mice, and solid bars are for CF mice. Similar data have been published previously (63). * \( P < 0.05 \), ** \( P < 0.01 \).
mucosa ($ctfr^{tm1Unc}$) exhibit a vigorous Cl$^-$ secretory response to the drug that is of similar magnitude to the forskolin response in the normal nasal mucosa (63) (Fig. 6B). Therefore, in the normal murine nasal mucosa, CFTR is the dominant Cl$^-$ secretory pathway. In CF nasal mucosa that express no CFTR, there is an upregulation of the Ca$^{2+}$-mediated Cl$^-$ secretory pathway. Others have confirmed these observations in vivo (nasal PD) for the $ctfr^{tm1Unc}$ and $ctfr^{tm1Hgu}$ CF mice (124). It is likely that the small response to forskolin in the CF nasal mucosa (in bilateral Krebs Ringer solution) (63) is due to a cAMP-induced increase in intracellular Ca$^{2+}$ (see tracheal data in sect. III). In cultured murine nasal epithelia from CF animals, we found no response to forskolin (21). Interestingly, the data from freshly excised nasal mucosa (and trachea) differ both qualitatively and quantitatively from those obtained from cultured nasal epithelia with respect to Ca$^{2+}$-mediated Cl$^-$ secretion, i.e., freshly excised nasal epithelia from normal mice respond to ionomycin with a Cl$^-$ secretory response of the same magnitude as that exhibited by the CF tissue (21).

The freshly excised nasal mucosa of the CF mouse thus appear to be an excellent model for human CF airway tissue, since this tissue exhibits both hyperabsorption of Na$^+$ and a defect in cAMP-mediated Cl$^-$ secretion, both characteristic of human CF airways. Although the predominant cell type in murine nasal mucosa, like human airway epithelium, is the ciliated cell (68), it should be pointed out that ~40% of the mucosal surface is lined by olfactory epithelia and most of the remainder by respiratory epithelium (67). In the human nasal cavity, >95% is composed of respiratory epithelium. Nevertheless, in studies of murine nasal tissue examined histologically after Ussing chamber studies, both the olfactory and respiratory epithelia from the CF mouse exhibited the Cl$^-$ transport defect as well as Na$^+$ hyperabsorption (Grubb, unpublished data).

4. Distal airways

In contrast to the human lower airways, which are composed primarily of ciliated cells, the murine lower airways (trachea, bronchi) contain >50% Clara cells (68). The ion transport physiology of the CF murine lower airways appears completely unlike that of the nasal mucosa, perhaps reflecting in part differences in the distribution of cell types.

In contrast to the nasal epithelia, only 30–70% of the basal $I_{sc}$ of the normal murine trachea appears to reflect Na$^+$ absorption (25, 36, 61, 73, 113); the remainder appears to reflect Cl$^-$ secretion. [This may reflect differences in mouse strains, because we have seen substantial differences in the magnitude of the amiloride-sensitive $I_{sc}$ among strains of mice (unpublished observation).] The first striking difference between the upper and lower mu-
There are several possible candidates for an apical Cl⁻ conductance in CF cells. The outward-rectifying Cl⁻ channel (ORCC) has been shown to be molecularly distinct from CFTR and is present in CF murine airway epithelia (53). However, this channel appears to be recognized only in excised membrane patches in murine tracheal epithelia, and its regulation by cAMP/protein kinase A has also been found to be defective in murine (53) as well as human (51, 89, 110) CF epithelial cells. Therefore, the cAMP-mediated Cl⁻ secretion in murine tracheal airway (and to a much lesser extent nasal epithelia) is not likely to be via the ORCC.

A study was then undertaken to determine the origin of the cAMP-mediated Cl⁻ secretory response in the CF trachea (61) in which intracellular Ca²⁺ measurements were made on fura 2-loaded (an intracellular Ca²⁺ indicator) cells from freshly excised murine tracheas and cultured murine tracheal cells (cftr tm1Bay). In both normal and CF cells from freshly excised murine tracheas, forskolin induced an increase in intracellular Ca²⁺, which was similar in magnitude for the two groups. Therefore, the forskolin-evoked Cl⁻ secretory response both in CF (totally) and normal tracheal preparations (at least partially) appears to be Cl⁻ secretion through an intracellular Ca²⁺-mediated non-CFTR pathway. In contrast, there was no forskolin-stimulated rise in Ca²⁺ in the cultured tracheal cells from either normal or CF animals (61), which may explain the absence of forskolin-stimulated Cl⁻ secretion in CF cultured monolayers. The reason that forskolin increases intracellular Ca²⁺ in the freshly excised preparation and not in cultured cells is not known. It may be that the culture conditions alter the intracellular signals such that the “cross talk” between cAMP and intracellular Ca²⁺ is abolished, e.g., protein kinase A-mediated sensitization of the inositol 1,4,5-trisphosphate receptor (17).

In the murine trachea (both cultured cells and freshly excised), most studies report no difference in the rate of Cl⁻ secretion between CF and normal preparations when tracheas are stimulated with agents that increase intracellular Ca²⁺ (ATP, ionomycin, A-23187) (21, 22, 61, 73, 113). However, for two of the mouse models (cftr tm2Unc and cftr TgHm1G551D), it has been reported that there is an upregulation of the Ca²⁺-mediated Cl⁻ secretory pathway in the CF tracheal epithelium (25, 36). It is likely that in the murine trachea, the alternative Cl⁻ secretory pathway is much more dominant than the cAMP-mediated CFTR pathway under basal and stimulated conditions, that the alternative Ca²⁺-mediated Cl⁻ secretory pathway (ClA) is not defective in CF, and that in some cases ClA is upregulated in CF airway tissue.

Two studies have examined fetal murine CF trachea, and in both of these studies (cftr tm1Bay and cftr tm1Unc), it was noted that both the normal and CF preparations responded to an increase in intracellular cAMP (forskolin or terbutaline induced) with an identical Cl⁻ secretory response (5, 92). Interestingly, in the cftr tm1Unc fetal tracheas, the cAMP-stimulated Cl⁻ secretion was not accompanied by an increase in intracellular Ca²⁺ (5) as was found in adult murine CF tracheas. Thus Barker et al. (5) speculate that in the fetal airway there is a non-CFTR Cl⁻ secretory pathway that is not mediated through an increase in intracellular Ca²⁺. Furthermore, the activity of this pathway tends to decrease, whereas the activity of the ClA tends to increase as the mouse pups mature (5). Others have also reported an increase in the activity of the ClA in murine airways as the mice mature (25).

To summarize the pulmonary phenotype in the various CF mouse models, the absence of pathology in the lower airways is a consistent finding among models. The upper airways of the various CF mice exhibit some relatively minor pathology. Furthermore, this region is functionally characterized by Na⁺ hyperabsorption and, in most models, an absence or marked decrease in cAMP-mediated Cl⁻ secretion. No significant Na⁺ hyperabsorption is noted in the tracheas of any of the CF mouse models. Furthermore, all of the mice exhibit a very prominent ClA and a significant Cl⁻ secretory response to forskolin. There are several possibilities as to why the CF mice are devoid of significant airway pathology. We have previously speculated that the prominent activity of the ClA in murine airway epithelia is able to replace the defective CFTR (which seems to have a small role in murine airway epithelium normally) and thus protects this tissue from disease (22). Indeed, we have noted an inverse correlation between the presence of disease pathology and activity of the ClA (see sect. III, A2 and D2) (22). Because it has been speculated that hyperabsorption of Na⁺ plays a role in the airway pathology of the human CF patient (80), another possible explanation for the lack of disease in CF murine lower airways is the lack of Na⁺ hyperabsorption in this tissue. A third possibility is that murine airways (with the exception of the very proximal trachea) lack submucosal glands (95), which in the human CF patient have been implicated in the pathology of CF airways (123). This possibility seems unlikely, however, because in the human CF patient, airway pathology is first manifested in the most distal airways (13), which are devoid of glands.

C. Hepatobiliary

1. Histopathology

High levels of CFTR mRNA are localized to the epithelial cells lining the human bile ducts (24), with little mRNA detectable elsewhere in the liver. A relatively large number of adult CF patients (20–50%) exhibit some form of hepatobiliary disease, ranging from mild to severe and either focal or multilobar (see Ref. 14 for review).

In the normal mouse, CFTR mRNA is detectable in

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Some of the gallbladders of the G551D CF mice have also been reported to be decreased in size (36).

2. Physiology

The gallbladder of several of the CF mouse models has been studied in Ussing chambers. We have found that the gallbladder of the normal mouse exhibits almost an identical Cl⁻ secretory response to agents that increase intracellular cAMP (forskolin) or intracellular Ca²⁺ (UTP) (Fig. 7). The cftr<sup>tm1Unc</sup> CF mouse, however, exhibits almost no forskolin response and a slightly although not significantly larger Cl⁻ secretory response to UTP than normal mice (Fig. 7). The cftr<sup>tm1Cam</sup> CF mouse follows a similar pattern characterized by an absence of a I<sub>sc</sub> forskolin response in gallbladders (99). The cftr<sup>tm1Eur</sup> CF mouse also exhibits a significantly decreased response on I<sub>sc</sub> response of normal or CF (cftr<sup>tm1Unc</sup>) gallbladders studied in small-aperture (0.025 cm<sup>2</sup>) Ussing chambers. Forskolin elicits a change in I<sub>sc</sub> (PD) to forskolin and a normal PD response to carbachol (a Cl⁻ secretory response) only in normal gallbladders. In contrast, UTP, by activating intracellular Ca²⁺-activated Cl⁻ channel Cl₂, stimulates a Cl⁻ secretory response in both normal and CF tissue. Although UTP response is somewhat larger in CF gallbladders, this response does not differ statistically from UTP response exhibited by normal gallbladders. Data shown are means ± SE, with sample size indicated in parentheses. * P < 0.01 vs. forskolin response by normal tissue.

The liver (99) but is expressed at higher levels in the gallbladder epithelium (98, 99, 114). In most of the CF mouse models for which there are data (70, 78, 92, 98, 114, 119), there appears to be no obvious liver pathology. However, some of the mice studied were young; animals may develop hepatic involvement as they age, as is characteristic of the human disease. In the G551D CF mouse (36), 20% of the mice are reported to exhibit hyperplasia of the bile duct epithelium.

In normal hepatobiliary ductal epithelia, the hormone secretin induces HCO₃⁻ secretion. In rat biliary ductal epithelium, it is thought that electrogenic Cl⁻ secretion is via CFTR (47). The Cl⁻ is then thought to exchange with cytosolic HCO₃⁻ by means of an apical Cl⁻/HCO₃⁻ exchanger. If this mechanism occurs in the mouse, it would be expected that there may be an inability to secrete HCO₃⁻ in the CF biliary ductal epithelium. The absence of major hepatobiliary disease in CF mice suggests a pathway of anion secretion that may differ from that seen in the rat. Obviously, this is an important area, and much work remains to determine the mechanism of anion secretion across murine hepatobiliary ductal epithelium.

The CF mouse gallbladder appears to exhibit more abnormalities than seen in the liver. However, the pathology is quite variable. The gallbladders of several of the CF mouse models have been reported to be distended (36, 92, 114) and filled with black bile (36, 114). The gallbladder wall of the cftr<sup>tm1Unc</sup> and the G551D CF mice have been noted to be infiltrated with polymorphonuclear cells, suggesting an ongoing inflammatory process (36, 114).
bladder cells (49). Despite the markedly reduced activity of the CFTR channels of the ΔF508 gallbladder epithelial cells, the biophysical signature (single-channel conductance) of the CFTR channel was identical to that of normal CFTR (49).

Cultured gallbladder epithelial cells secrete high-molecular-weight glycoproteins, approximately one-third of which is mucin (98). Study of mucin secretion by gallbladder may be instructive, because biliary disease in human CF patients appears to result at least in part from obstruction of the biliary ducts with mucus. In murine gallbladder cells, neither normal nor cftr<sup>tm1Unc</sup> CF mice exhibited an increase in glycoprotein secretion with an increase in cAMP, Ca<sup>2+</sup>, or protein kinase C (98). Furthermore, there was no significant difference in the endogenous rate of high-molecular-weight glycoprotein secretion between the normal and CF murine gallbladder cells (98). Thus the data do not show a clear relationship between CFTR function and mucin secretion by gallbladder epithelial cells. However, it remains to be determined whether the glycoprotein composition from the CF gallbladders differs from normal. This is especially important because in human CF patients it has been found that the glycoproteins exhibit an increased sialation and sulfation as well as abnormal carbohydrate structure (18).

D. Pancreas

In the pancreas, the acinar epithelia secrete digestive enzymes, and the CFTR-expressing ductal epithelia secrete a HCO<sub>3</sub>-rich liquid that flushes the enzymes into the duodenum. In the human CF patient, plugging of the pancreatic ducts with mucus leads to.insipissated luminal proteins. The acinar epithelia continue to secrete digestive enzymes, which accumulate in the acini due to the blocked ducts, leading to enlarged acini, autolysis of the acini, and eventual replacement of the acini with fibrotic tissue. It is for this pathophysiological process that the disease was named (cystic fibrosis of the pancreas). Compared with the relatively severe pancreatic pathology observed in human patients, the pathology reported for the various CF mouse models appears to be much less severe.

1. Histopathology

None of the ΔF508 models or the G551D model exhibits any obvious pancreatic pathology (25, 36, 119, 131). The cftr<sup>tm1Hgu</sup> mouse (39) exhibits no pancreatic pathology, probably as a result of expression of a significant amount of wild-type CFTR (see below). The knockout models appear to exhibit more, although variable, pancreatic pathologies. In the cftr<sup>tm1Unc</sup> mouse, two of five mice examined exhibited enlarged acini containing eosinophilic material in one or two lobes of the pancreas (114). Another report noted that the acini of these mice had dilated lumens filled with amylase (37). Interestingly, in contrast to the human CF pancreas, the ductal structures in this CF mouse model appeared normal (37). In the normal acini, pancreatic amylase was found located in the zymogen granules, membrane-bound organelles containing the digestive enzymes. At the ultrastructural level, the acini of these CF mice contained few zymogen granules, and their diameter was about one-half that seen in the normal murine pancreatic acini (37). In this study, it was also noted that the levels of pg300, the major sulfated glycoprotein in the mouse acinar cell (and thought to function in the biogenesis of the zymogen granules), were significantly elevated in the acini of the CF mouse (37). It was noted, however, that there were no apparent changes in sulfate or carbohydrate composition of the pg300 glycoprotein (37). This is important because in the human CF patient, it has been reported that secretory glycoproteins have greater than normal sulfate content (18). Another group examining the same mouse model noted no pancreatic pathology in older CF mice (78).

The cftr<sup>tm1Cam</sup> CF mouse was reported to exhibit blockage of some of the small pancreatic ducts in ~50% of the mice examined, although the lesions were not considered severe enough to alter pancreatic function (103). The two CF mouse models developed at Baylor University exhibited a different type of acinar pathology. These mice were found to have acinar atrophy that appeared to progress as the mice aged (70, 92). One 6-wk-old CF mouse exhibited severe atrophy of the entire pancreas with mild dilation of the ducts (92). The authors suggest that these changes may be related to the poor nutritional status of these mice. No pathology was noted in the islet cells.

2. Physiology

One group examined the exocrine pancreatic function in the cftr<sup>tm1Unc</sup> mouse kept on a liquid diet to increase longevity. This group noted that although the longevity of the CF mouse was increased by the diet (see sect. IIIA), the CF animals still exhibited a significantly lower body mass and pancreatic mass compared with control animals (74). Furthermore, pancreatic protein content and the activity of two pancreatic enzymes (amylase and lipase) were significantly lower than in age-matched controls (74). It was noted, however, that the lowered pancreatic enzyme levels may simply be a result of malnutrition, because it has been found that both lipase and amylase levels are affected by malnutrition in the rodent (74). This explanation seems probable because it has been noted that >90% of the pancreas can be destroyed without any noticeable change in pancreatic function (see Ref. 92). Furthermore, the cftr<sup>tm1Hgu</sup> CF mouse, which exhibits no malabsorption or other major gut problems, did not show a decrease in pancreatic amylase secretion in vitro (90). (However, it needs to be stressed that this CF mouse model also exhibits some wild-type CFTR; see sect. II.)
Electrophysiological investigations have been carried out on murine pancreatic ductal cells. Both whole cell patch-clamp (57, 125) and Ussing chamber studies (22) suggest that in normal murine pancreatic ductal cells the Ca$^{2+}$-activated Cl$^{-}$ conductance dominates over the CFTR Cl$^{-}$ conductance (Fig. 9). These data are also consistent with the low levels of CFTR mRNA detection in murine pancreas (114), whereas in the normal human pancreas, CFTR mRNA is expressed at much higher levels. No cAMP-activated Cl$^{-}$ conductance was present in pancreatic ductal cells from the ΔF508 CF mouse (131). The pancreatic ductal cells from the ΔF508 CF mouse (131) failed to secrete liquid in response to agonists that raise intracellular Ca$^{2+}$, whereas the normal pancreatic cells responded to the drug cocktail with a significant secretory response (131). The CF mouse model generated by targeted insertional mutagenesis of exon 10 (cflr$^{tm1Kth}$) exhibited CFTR current densities from whole cell patch-clamped pancreatic ductal epithelium that were reduced by only ~50% compared with normal, again undoubtedly reflecting the presence of functional CFTR (57).

In ductal cells from the ΔF508 CF mouse, the amiloride-sensitive $I_{sc}$ did not differ significantly between normal and CF pancreatic cells (131). This contrasts with data from the CF murine nasal and distal colonic epithelia in which it has been found that the amiloride-sensitive $I_{sc}$ is significantly increased. However, tracheal epithelium of the CF mouse appears to be similar to the pancreas in that there is no upregulation of Na$^{+}$ absorption in CF tissue. Interestingly, both of the tissues (nasal and colon) exhibiting CF-specific hyperabsorption of Na$^{+}$ have a very prominent cAMP-mediated Cl$^{-}$ secretory response in normal tissue and little or no Ca$^{2+}$-mediated Cl$^{-}$ secretory response. In contrast, the two CF tissues exhibiting no upregulation of Na$^{+}$ absorption (trachea and pancreas) exhibit very little cAMP-mediated Cl$^{-}$ secretion and a very dominant Cl$^{-}$ conductance in normal tissue. Studies on the relationship between amiloride-sensitive Na$^{+}$ absorption and CFTR function in these tissues may therefore provide additional insight into the relationship between these two proteins.

It has been suggested that in the murine pancreas the presence of the very dominant Ca$^{2+}$-mediated Cl$^{-}$ conductance in both normal and CF pancreatic ductal cell is able to compensate, at least in part, for the loss of CFTR; thus the pathology of the murine CF pancreas is much milder than that of its human counterpart (22, 57).

### E. Reproductive Tissue

#### 1. Male pathophysiology

Most male human CF patients are infertile because of obstruction and/or atresia of the vas deferens and distal epididymis (71). This obstruction is thought to be because of the presence of dehydrated secretions, likely reflecting the absence of cAMP-mediated electrolyte and fluid secretion in these structures. In contrast, conception is possible in the human CF female patient, although fertility is reduced (93).

Despite the fact the normal male mice are reported to express relatively high levels of CFTR mRNA in the testes and epididymis (114), virtually no pathology of the murine CF reproductive tract has been reported in any of the CF mouse models. The males all appear to be fertile (36, 39, 70, 92, 114, 119, 131) and sire normal-sized litters when mated to normal females (cflr$^{tm1Unc}$). In a study of cultured primary normal and CF (cflr$^{tm1Unc}$) murine epididymides and seminal vesicles, it was found that normal tissue exhibited a cAMP-mediated (forkolin) Cl$^{-}$ secretory response, which was lacking in the CF testes (87). However, agents that raise intracellular Ca$^{2+}$ (ionomycin and ATP) elicited a larger Cl$^{-}$ secretory response in normal tissue than did forskolin, and this Ca$^{2+}$-mediated Cl$^{-}$ secretion persists to the same level in the male reproductive tissue from the CF mice (87). Therefore, it was speculated that the fertility in the male CF mouse is maintained by the presence of the predominant Ca$^{2+}$-mediated Cl$^{-}$ secretory pathway in the epididymides and seminal vesicles.

#### 2. Female pathophysiology

In the mouse models for which data are reported, there appears to be no pathology present in the female reproductive tract (36, 39, 78, 114, 131). However, de-
spite the seemingly normal histology of the female reproductive tract of the knockout CF mouse models, these mice exhibit a markedly reduced fertility (70; Koller, personal communication). Although pregnancy in the cftr<sup>tm1Unc</sup> mouse has been achieved, a much greater length of time is needed to achieve conception, and litter sizes are very small (Koller, personal communication). The female cftr<sup>tm1Kth</sup> CF mouse appears to exhibit normal fertility (131).

In a study on primary cultures of murine oviductal epithelium from normal and CF (cftr<sup>tm1Unc</sup>) mice, it was found that normal tissue exhibited a cAMP-mediated Cl<sup>-</sup> secretory response that was absent in the CF oviductal epithelium (86). Like the male reproductive tissue, the oviductal epithelium from normal mice exhibited an ATP-stimulated Ca<sup>2+</sup>-mediated Cl<sup>-</sup> secretory response, and the magnitude of the large Ca<sup>2+</sup>-activated response was maintained in the CF tissue (86). Therefore, as in the male reproductive tissue and other epithelial tissues in the CF mouse lacking pathology, the murine oviduct may be protected against the loss of CFTR by the presence of a prominent Ca<sup>2+</sup>-mediated Cl<sup>-</sup> secretory pathway. Because the murine uterus expresses relatively high levels of CFTR mRNA (114), perhaps the very low pregnancy rate in the CF mouse may be related to defects in implantation due to an unfavorable uterine environment. Alternatively, the marked reduction in fertility of the female CF mouse may be a result of an unfavorable vaginal or cervical environment that inhibits normal sperm motility.

**F. Salivary Glands**

1. **Pathophysiology**

There is good evidence that in salivary glands both β-adrenergic stimulation, which increases cAMP levels, and stimulation by such agents as substance P and acetylcholine, which increases intracellular Ca<sup>2+</sup>, can induce salivary gland secretion (26). The electrolyte composition of the primary saliva (produced by the acini) in general has an isotonic plasmalike electrolyte composition. As the saliva passes through the ducts, the ionic composition is markedly modified by the absorption of NaCl and the secretion of KHCO<sub>3</sub>. [Saliva recovered from the duct of the normal murine submaxillary gland fits this pattern because the Na<sup>+</sup> and Cl<sup>-</sup> concentrations were very low, 19.1 ± 6 and 5.8 ± 5.8 mM (<i>n</i> = 6), respectively, whereas the K<sup>+</sup> concentration was very high, 69.3 ± 19.5 mM (<i>n</i> = 6); unpublished observations.]

The normal murine salivary gland expresses high levels of CFTR mRNA (114, 131), localized to the serous gland collecting ducts (70). These ducts are thought to play a major role in electrolyte transport (70). A recent report has by immunolocalization identified CFTR in both the ducts and acini of the submandibular gland of the normal mouse. No CFTR was identified in either structure of the CF mouse (132).

In the human CF patient, submaxillary and sublingual pathology includes dilated ducts, inspissated secretions, and atrophy of the acini (93). No pathology was noted in the salivary glands of the ΔF508 CF mouse models (119, 131). In the cftr<sup>tm1Unc</sup> CF mouse, the submaxillary glands show varying degrees of disrupted serous acini (114). This mouse model did not exhibit dilation of the ducts or the presence of inspissated material in the ducts. Acinar and ductal tissue in the cftr<sup>tm1Hpo</sup> CF mouse were indistinguishable from normal (90). In another of the knockout CF mouse models (cftr<sup>tm1Bay</sup>), severe dilation of the acini of the minor sublingual gland was reported in young mice, but no other pathology in the main salivary gland was noted (92). However, in older animals (>6 wk), parotid gland atrophy was noted (92). The salivary gland of the G551D CF mouse model (cftr<sup>TgHmlG551D</sup>) appears to be hypercellular because of the serous cells having lost their vacuolated appearance (36).

Although little physiology of CF murine salivary glands has been reported, we have studied the effect of isoproterenol, an agent that increases intracellular cAMP levels, on the salivary secretory rate from the submaxillary gland in normal and CF mice (cftr<sup>tm1Unc</sup>). This preliminary study revealed that when the submaxillary gland of the mouse was stimulated with isoproterenol, the flow rates in normal mice were more than double those measured in the CF mouse (Fig. 10). Similar data have been described for the human CF patient (33). The reduced salivary secretory rate we observed (Fig. 10) in the CF murine submaxillary gland likely indicates that the normal submaxillary gland secretes in response to both an in-
crease in cAMP (isoproterenol) and an increase in cellular 
Ca\(^{2+}\), possibly as a result of endogenous acetylcholine 
production. In the CF salivary gland, the ability to respond 
to cAMP (through CFTR) would be eliminated, and only the 
Ca\(^{2+}\)-mediated secretory pathway would remain; thus 
the secretory rate would be diminished. In a study of glycoprotein secretion by submandibular gland tissue in 
vitro in response to isoproterenol in the cftr\(^{tm1Unc}\) mouse, it was noted that the tissue from the CF animals exhibited 
a significantly reduced glycoprotein secretory response 
compared with wild-type controls (90).

G. Teeth

1. Pathophysiology

We have made the observation that the incisor teeth of CF mice (cftr\(^{tm1Unc}\)) are strikingly different in appearance 
from the incisors of normal animals. All CF mice examined have soft, chalky white, easily fractured incisor enamel, whereas the enamel of normal mice is hard and yellow-brown in color (127). [The cftr\(^{tm1Cam}\) and cftr\(^{tm1Bay}\) mice also exhibit this defect (127).] The lack of tooth pigmentation is the result of abnormal enamel development and does not reflect post eruption phenomena or a salivary influence (127). Light microscopy studies revealed that the ameloblasts, the cells responsible for the enamel formation, appear to undergo premature degeneration in CF mice (127). The enamel of the CF incisors is hypomineralized, but the Ca\(^{2+}\)-to-phosphate ratio is normal. Interestingly, the Mg\(^{2+}\) content of the CF enamel is elevated (126). Although we cannot rule out the possibility that the nutritional status of the CF mice influences enamel development, we have noted that the serum calcium, phosphate, and iron levels do not differ between normal and CF animals (127). Furthermore, the same phenotypic incisor abnormalities are present in a CF mouse model that does not exhibit severe gut disease (cftr\(^{tm1Unc}\)) (P. French, personal communication). In addition, in the gut-corrected CF mouse (133) (see sect. IIIA), the incisor abnormality persists (Jackson Labs, personal communication). On a practical note, these CF mice can be easily distinguished from their heterozygote or wild-type littermates as early as 3 wk of age by the phenotypic incisor abnormalities.

Numerous reports describe enamel defects in human patients, but clearly many of these developmental defects have been associated with tetracycline therapy, confounding the exact etiology of the enamel defects. However, it appears that some human CF patients exhibit hypomineralized enamel, described as white areas in the tooth enamel (100) similar to what has been observed in the CF mice.

IV. GENETIC MODULATION OF DISEASE SEVERITY

The cftr\(^{tm1Hsc}\) CF mouse, like many of the other CF mouse models, exhibits no CFTR function, severe intestinal pathophysiology, and early death in most CF mice (107). However, a small subset of these CF mice (class III mice) seem to exhibit normal survival and body mass at maturity despite having no CFTR-mediated Cl\(^{-}\) secretion in the intestinal tract (107). Strong evidence from genetic linkage analyses indicates the presence of modifying loci localized to chromosome 7 that modulate the severity of the disease in these class III CF mice.

The investigators provide physiological evidence suggesting that the intestinal tract of these mice may express an alternative Ca\(^{2+}\)-mediated Cl\(^{-}\) conductance, Cl\(_\lambda\), that may compensate for the absence of CFTR. In a whole cell patch-clamp study of ileal crypt cells, it was found that there was no Ca\(^{2+}\)-mediated Cl\(^{-}\) conductance in cells from the normal cftr\(^{tm1Hsc}\) mice, whereas the class III CF mice exhibited a Cl\(^{-}\) secretory current in response to the Ca\(^{2+}\) ionophore A-23187 (107). However, evidence was not provided that this Cl\(^{-}\) current activity was due to an apical membrane Cl\(^{-}\) channel.

In another investigation of these class III mice, it was found that UTP evoked a significant increase in rectal PD (thought to be Cl\(^{-}\) secretion), whereas normal mice or the cftr\(^{tm1Unc}\) CF mouse failed to exhibit a significant rectal PD response to UTP perfusion (124). These investigators suggest that these data further support the presence of a Ca\(^{2+}\)-mediated Cl\(^{-}\) secretory pathway in the intestinal tract of the class III cftr\(^{tm1Hsc}\) mice (124). Clearly, genes capable of modifying CF disease severity are of great interest, and this mouse model may play a fundamental role in identifying the responsible genes.

V. GENE THERAPY

Studies have employed four of the CF mouse models for gene therapy, using either liposomal or adenoviral vectors. Perhaps the one most significant conclusion from these studies is that the CF mouse (especially when adenoviral vectors were used) accurately predicted results in human clinical trials, i.e., gene transfer efficiency to airway epithelium is low (see sect. vB).

A. Liposomal Vectors

Both the cftr\(^{tm1Cam}\) and the cftr\(^{tm1Hgu}\) mouse models were employed in liposome-mediated gene transfer trials. In the study using the cftr\(^{tm1Cam}\) mouse, the human CFTR cDNA encoding the entire CFTR protein was inserted into the vector pREP8, the plasmid complexed with cationic liposomes, and delivered in vivo by direct tracheal instilla-
tion (73). Four days later, the tracheas were removed from CF and control mice and studied in Ussing chambers. The defect in cAMP-mediated Cl\(^-\) secretion was partially corrected in the tracheas of the CFTR-treated CF mice. In addition, the rather unique hypoabsorption of Na\(^+\) reported in this CF mouse model was corrected. The Ca\(^2+\)-mediated Cl\(^-\) secretory response (induced by A-23187) was paradoxically elevated by the CFTR gene therapy (73). It has been noted (1) that it is unclear how transfection with CFTR cDNA could correct such a range of transport properties.

Another group of investigators nebulized a cocktail of CFTR cDNA expression plasmids complexed to a cationic liposome into the airways of the cftrtm1Hgu CF mouse (1). The nasal PD technique was employed to test for correction of the ion transport defects in the nose. The nasal PD technique adequately discriminates CF from the normal phenotype based on both “CF-specific” hyperabsorption of Na\(^+\) and a defective cAMP-mediated Cl\(^-\) secretion (62) (see Fig. 5). The nasal PD measurements revealed a 50% correction in the Cl\(^-\) transport defect; however, the magnitude of this correction failed to reach statistical significance. No correction in the hyperabsorption of Na\(^+\) across the nasal epithelia of the CF mouse was noted (1). Tracheas were removed from the treated mice and studied in vivo on Ussing chambers. The authors reported that in some of the CF animals a complete correction in the Cl\(^-\) transport defect was seen in both the nasal and tracheal preparations (1). Although these studies suggested that the treatment raised the cAMP-mediated Cl\(^-\) secretory response in the tracheas of the CF animals, the data failed to achieve statistical significance. As in the liposomal-mediated correction in the CF (cftrtm1Cam) mouse, the low amiloride-sensitive Na\(^+\) transport reported in the tracheas of the CF (cftrtm1Hgu) mice shifted toward normal. However, because it has been convincingly demonstrated that CFTR downregulates amiloride-sensitive Na\(^+\) absorption (115), the mechanism for this finding is unclear.

**B. Adenoviral Vectors**

The efficacy of adenoviral-mediated CFTR gene transfer in vivo to the nasal epithelium of the CF mouse (cftrtm1Huc) was studied using a complementary series of molecular (in situ hybridization, immunocytochemical) and functional (PD) techniques (62). Despite the finding of full functional correction of cultured CF human airway epithelium in vitro using an adenovector (75, 128), in vivo delivery of the adenoviral vector containing human CFTR to CF mouse nasal epithelium resulted in much less efficient gene transfer. A single dose of vector failed to restore Cl\(^-\) transport to normal. However, mice treated with the high dose vector 4 days in succession exhibited a significant correction (50% of normal) of the Cl\(^-\) transport defect, which was associated with only a small fraction (<3%) of the dosed nasal cells expressing the transgene (62). This study also demonstrated that the restoration of Cl\(^-\) transport was transient, and the correction had waned by day 10 postdosing. Like the mice treated with CFTR complexed with liposomes, there was no downregulation in the nasal hyperabsorption of Na\(^+\) in the nasal tissue of the CF mice treated with adenovirus serotype 5 CMV enhancer B-actin promoter (62). Perhaps the most important finding from this investigation, later borne out in human clinical trials (83, 129), is that adenoviral gene transfer to fully differentiated epithelial cells in vivo is very inefficient. A more recent study on adenoviral-mediated CFTR gene transfer (Ad2/CFTR-8) to the nasal epithelium of CF mice (cftrtm1Rhr) confirmed the findings of very inefficient gene transfer to ciliated nasal epithelia (130). However, it was noted that if the contact time between vector and nasal epithelia was increased, better gene transfer efficiency resulted (130).

**VI. PHARMACOTHERAPY**

Although the various CF mouse models do not appear to exhibit lung disease, this feature nevertheless does not prevent functional testing in these mice of various pharmacological agents aimed at lung disease. Because the mice exhibit such a predominant Ca\(^2+\)-mediated Cl\(^-\) secretion in the lower airways, they provide a good opportunity to test drugs such as UTP that activate this Cl\(^-\) conductance and have been proposed as possible therapeutic agents in the human CF patient (82). The Cl\(^-\) secretory response to available drugs that activate Cl\(_A\) is usually very transient; testing pharmacokinetics in mice provides a good opportunity to study compounds that may have a more prolonged duration of action.

Because it has been suggested that the hyperabsorption of Na\(^+\) may contribute to the airways disease in human CF patients, and as all CF mouse models exhibit hyperabsorption of Na\(^+\) across the nasal epithelium, these mice will be important in testing various drugs to reduce this elevated rate of Na\(^+\) absorption. The elevated nasal Na\(^+\) absorption in CF mice has been demonstrated to be amiloride sensitive (see sect. III). One study has used the CF mouse (cftrtm1Hgu) to compare the efficacy of loperamide to amiloride in blocking elevated Na\(^+\) transport across the nasal epithelium (54).

Perhaps the ΔF508 mouse models will be most important in testing therapeutic agents. This speculation reflects the fact that ΔF508 is the most common mutation in the human population, being responsible for >70% of mutations. In the human ΔF508 mutation, CFTR is not processed to its fully glycosylated form, the mutated protein being retained in the endoplasmic reticulum until degradation occurs (20). Thus little, if any, ΔF508 protein is...
inserted into the apical membrane, and as a result, epithelial tissues from these patients do not exhibit a cAMP-mediated Cl\(^-\) conductance. However, if the \(\Delta F508\) protein can be inserted into the apical membrane, the mutated protein appears to be able to function as a CAMP-regulated channel (30, 38). Some studies report that \(\Delta F508\) CFTR has near-normal function as a Cl\(^-\) channel compared with wild-type CFTR (49, 88, 97). Others have noted a decrease in open-channel probability with the \(\Delta F508\) channel (30).

It has been demonstrated that reducing the temperature at which \(\Delta F508\)-expressing cells are cultured (from 37 to \(<30^\circ C\)) can in part overcome the trafficking defect, allowing the \(\Delta F508\) CFTR to insert into the apical membrane and function as a CAMP-regulated Cl\(^-\) channel (38). In human \(\Delta F508\) CF cells in vitro, there are also other strategies that are effective in overcoming the processing defect. Compounds, e.g., glycerol, stabilize the immature \(\Delta F508\) protein and appear to allow some \(\Delta F508\) protein to insert into the apical membrane (109). Another strategy that is effective in human \(\Delta F508\) airway tissue in vitro is treatment of the tissue with sodium butyrate, which by unknown mechanisms allows an increased trafficking of the mutated protein to the apical membrane of these CF cells (19). These compounds warrant testing in the \(\Delta F508\) CF mouse.

If it could be demonstrated that the murine \(\Delta F508\) CFTR protein undergoes the same processing defects as does its human counterpart, then the \(\Delta F508\) mouse model would be of enormous benefit for testing some of these treatment strategies. Indeed, it has been shown that airway cells from the \(cft\text{r}_{\text{mut2c}}\text{mut}\) mouse model exhibit a temperature-sensitive CFTR trafficking defect. Tracheal cells from these \(\Delta F508\) mice exhibited almost no anion efflux (SPQ assay) in response to stimulation with cAMP agonists when the cells were cultured at 37\(^\circ C\). In contrast, when the airway cells were cultured at 27\(^\circ C\), a CAMP-mediated anion efflux was detected (25). In the \(cft\text{r}_{\text{tm1Kth}}\) \(\Delta F508\) mouse, similar conclusions were drawn from data obtained from gallbladder cells studied by the patch-clamp technique (49). Taken together, the data from these two studies indicate that the \(\Delta F508\) mouse models exhibit a processing defect similar to that seen in \(\Delta F508\) human tissue.

Because the \(\Delta F508\) protein in cultured human cells has been shown to be activated by a combination of a class III phosphodiesterase inhibitor (milrinone) and forskolin, the in vivo effect of this cocktail on CAMP-mediated Cl\(^-\) transport across the nasal epithelia of the \(\Delta F508\) CF mouse (\(cft\text{r}_{\text{tm1Kth}}\)) was investigated (77). It was found that perfusing the nasal epithelia with Cl\(^-\) -free buffer containing the milrinone/forskolin mixture evoked a significant hyperpolarization of the electrical PD across the nasal epithelia of both the normal and the \(\Delta F508\) CF mouse, consistent with a Cl\(^-\) secretory response. In contrast, the nasal epithelia of the \(cft\text{r}_{\text{tm1Kth}}\) CF mouse (no functional CFTR protein present) failed to respond to the drug cocktail with a Cl\(^-\) secretory response. These results demonstrate that some functional CFTR is present in the nasal epithelia of this \(\Delta F508\) CF mouse and that by elevating the intracellular cAMP levels, at least partial CFTR-mediated Cl\(^-\) secretion can be restored.

### VII. FUTURE OF THE CYSTIC FIBROSIS MOUSE

The CF mouse models generated to date have provided a wealth of information on the pathophysiology of the disease in a variety of organs. Marked similarities to and differences from the human disease have been observed in the various murine models. Some of the murine models exhibit some functional CFTR, which provides the opportunity to study the correlation between phenotype and the quantity of functional CFTR present. Studies are just beginning to appear on the heterogeneity of disease severity and the presence of modifying genes.

Because the intestinal pathophysiology of most of the CF mouse models so closely mimics that of the CF human, the intestinal tract of these animals has provided much information on the pathogenesis of gastrointestinal dysfunction in CF patients as well as providing information on basic ion transport physiology. Although information has not been provided as to why the CF mice are underweight compared with their normal littermates, these animals should be useful for investigating nutritional therapy to enhance weight gain, which will be of benefit to most CF patients.

The excitement generated by the emergence of the first CF mouse models was tempered somewhat by the finding that these animals, unlike CF patients, do not spontaneously develop lung disease. However, by conclusively establishing why CF mice are not susceptible to pulmonary infections, important information will be provided regarding the pathogenesis of human CF airways infections. Various groups are presently attempting to establish airway infections by repeated bacterial exposure and the deposition of bacteria-impregnated agarose beads into the respiratory tract of CF mice. A CF mouse with pulmonary pathology that more closely mimics that of humans would have obvious benefits for developing effect therapeutic strategies to combat these infections. If the alternative Ca\(^{2+}\)-activated Cl\(^-\) secretory pathway, thought to protect the mice from airways disease, could be either pharmacologically or molecularly knocked out in the CF mice, this concept of a protective mechanism could be tested and a CF mouse model with airways disease generated. As various other genetically engineered mouse models are generated (P2y\(_2\) receptor knockout, mice devoid of submucosal glands, etc.), these animals then can be mated with CF mice to provide additional models useful
for studying the interactions of a variety factors on the pulmonary phenotype. Nevertheless, the murine CF airways, especially the nasal epithelia, have proven especially useful in gene therapy trials. The airways of the ΔF508 mice will be of utmost importance in testing various pharmacological protocols aimed at circumventing the CFTR trafficking defect in these mice.

There are numerous reports in the literature of cellular dysfunctions, e.g., Na⁺ channel regulation, ORCC regulation, ATP release, control of exocytosis/endocytosis, cell volume regulation, intracellular pH regulation, and trans-Golgi network acidification, resulting from loss of calcium but not protein kinase C- and A-dependent mechanisms.

As the various CF mouse models are further modified and refined to more closely mimic the human phenotype, especially with respect to airways disease, these animals should provide the missing information necessary to allow rapid development of more effective treatment and/or cure for this devastating disease.

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